

AD _____

Award Number: DAMD17-92-C-2066

TITLE: Evaluation of Biomonitoring Systems for Assessment of
Contaminated Waters and Sediments at U.S. Army
Installations

Subtitle: Toxicity of Diisopropyl Methylphosphonate (DIMP) to
Aquatic Organisms at the Building E3640 Process
Laboratory Site, Aberdeen Proving Ground-Edgewood Area,
Aberdeen, Maryland

PRINCIPAL INVESTIGATOR: Dennis T. Burton, Ph.D.
Steven D. Turley, M.S.

CONTRACTING ORGANIZATION: University of Maryland System
Agricultural Experiment Station
Queenstown, Maryland 21658

REPORT DATE: September 1999

TYPE OF REPORT: Final (Supplemental)

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release;
distribution unlimited

The views, opinions and/or findings contained in this report are
those of the author(s) and should not be construed as an official
Department of the Army position, policy or decision unless so
designated by other documentation.

20000517 044

DTIC QUALITY INSPECTED 3

REPORT DOCUMENTATION PAGEForm Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)**2. REPORT DATE**

September 1999

3. REPORT TYPE AND DATES COVERED

Final, Supplemental (18 Aug 97 - 30 Sep 98)

4. TITLE AND SUBTITLE

Evaluation of Biomonitoring Systems for Assessment of Contaminated Waters and Sediments at U.S. Army Installations

Subtitle: Toxicity of Diisopropyl Methylphosphonate (DIMP) to Aquatic Organisms at the Building E3640 Process Laboratory Site, Aberdeen Proving Ground-Edgewood Area, Aberdeen, Maryland

6. AUTHOR(S)

Dennis T. Burton, Ph.D.

Steven D. Turley, M.S.

5. FUNDING NUMBERS

DAMD17-92-C-2066

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)University of Maryland System
Agricultural Experiment Station
Queenstown, Maryland 21658
E-MAIL: db124@umail.umd.edu**8. PERFORMING ORGANIZATION
REPORT NUMBER****9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012**10. SPONSORING / MONITORING
AGENCY REPORT NUMBER****11. SUPPLEMENTARY NOTES****12a. DISTRIBUTION / AVAILABILITY STATEMENT**

Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE**13. ABSTRACT (Maximum 200 Words)**

Diisopropyl methylphosphonate (DIMP) is present in the surficial groundwater in the vicinity of the Building E3640 Process Laboratory at the U.S. Army Aberdeen Proving Ground-Edgewood Area, Aberdeen, Maryland. The acute and chronic toxicity of DIMP and the possible interactions of DIMP with other contaminants that may be present in the groundwater and the parent compound were both evaluated using the following bioassay systems: 96-h green algal (*Selenastrum capricornutum*) growth test; 7-d cladoceran (*Ceriodaphnia dubia*) survival and reproduction test; and 7-d larval fathead minnow (*Pimephales promelas*) survival and growth test. In addition, survival and developmental toxicity were determined by the 96-h frog embryo teratogenesis assay-Xenopus (FETAX) using the African clawed frog, *Xenopus laevis*. The groundwater, which contained DIMP concentrations up to 6.02 mg/L, was not acutely or chronically toxic to the alga, cladoceran, frog, or fish. The no-observed-adverse-effect levels (NOAEL) and lowest-observed-adverse-effect levels (LOAEL) for short-term chronic exposure to the parent compound were as follows: alga (reduction in growth) = 711 and 1,423 mg/L; cladoceran (reduction in neonate production) = 142 and 285 mg/L; and fish (reduction in growth) = 142 and 285 mg/L. The NOAEL and LOAEL for the frog embryo (mortality) = 398 and 569 mg/L.

14. SUBJECT TERMSDiisopropyl methylphosphonate, DIMP, groundwater, acute toxicity, chronic toxicity, algae, invertebrates, fish, FETAX, *Selenastrum capricornutum*, *Ceriodaphnia dubia*, *Xenopus laevis*, *Pimephales promelas*, priority pollutants, heavy metals, VOCs, munitions, NOAEL, LOAEL**15. NUMBER OF PAGES**

125

16. PRICE CODE**17. SECURITY CLASSIFICATION
OF REPORT**

Unclassified

**18. SECURITY CLASSIFICATION
OF THIS PAGE**

Unclassified

**19. SECURITY CLASSIFICATION
OF ABSTRACT**

Unclassified

20. LIMITATION OF ABSTRACT

Unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

___ Where copyrighted material is quoted, permission has been obtained to use such material.

___ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

✓ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

N/A In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

N/A For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

N/A In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

N/A In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

N/A In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

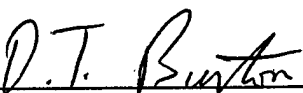
D.T. Burton 9-30-99
PI - Signature Date

Certification of Technical Data Conformity (May 1987)

The contractor, University of Maryland at College Park, hereby certifies that, to the best of its knowledge and belief, the technical data delivered herewith under Contract DAMD17-92-C-2066 is complete, accurate, and complies with all requirements of the contractor.

Date: September 30, 1999

Name and Title of Certifying Official:


Dennis T. Burton, Ph.D.
Senior Research Scientist

EXECUTIVE SUMMARY

Diisopropyl methylphosphonate (DIMP) is present in the subsurface soils and surficial groundwater in the vicinity of the Building E3640 Process Laboratory at the U.S. Army Aberdeen Proving Ground-Edgewood Area, Aberdeen, Maryland. DIMP has been found in two surficial wells downgradient of a surficial groundwater divide where the groundwater flow is to the north toward Kings Creek. The current study was initiated to determine the acute and chronic toxicity of DIMP and the possible interactions of DIMP with other contaminants that may be present in the groundwater as it enters the aquatic environment. The toxicity of DIMP and its interaction with other contaminants in the most highly contaminated well at the site (well # CCJ153A) was investigated as a worst case condition in the surficial aquifer north of the Building E3640 Process Laboratory. The toxicity of the parent compound was also determined to confirm the acute toxicity data in the literature and to provide chronic toxicity data which were not available.

The acute and chronic toxicity of DIMP and the possible interactions of DIMP with other contaminants that may be present in the groundwater and the parent compound were both evaluated using an array of four biomonitoring systems which included a number of endpoints. The biomonitoring assays covered several levels of biological complexity to maximize the predictability of DIMP toxicity to aquatic organisms. The following toxicity tests were conducted: 96-h green algal (*Selenastrum capricornutum*) growth test; 7-d cladoceran (*Ceriodaphnia dubia*) survival and reproduction test; and 7-d larval fathead minnow (*Pimephales promelas*) survival and growth test. In addition, survival and developmental toxicity were determined by the 96-h frog embryo teratogenesis assay-*Xenopus* (FETAX) using the African clawed frog, *Xenopus laevis*.

The concentrations of DIMP in the surficial groundwater samples taken from well CCJ153A on March 16, 18, and 20, 1998 at the Building E3640 Process Laboratory site, were 6.05, 5.09, and 4.72 mg/L, respectively. Low concentrations of several priority pollutant heavy metals (aluminum, barium, chromium, copper, and manganese) and one volatile organic (vinyl chloride) were also present in one or more of the three groundwater samples. No base neutrals, acid extractables, organophosphorus pesticides, or chlorinated pesticides and herbicides were found in the groundwater above the detection limits for drinking water. No nitroaromatic or nitramine explosives above a detection limit of 50 µg/L were present.

The groundwater was not acutely toxic to the green alga, cladoceran, or larval fish. The groundwater was not acutely toxic to frog embryos after 96 h of exposure. A statistically significant ($\alpha = 0.05$) effect was found for frog embryo malformations; however, the effect was judged to be statistical error because the concentrations in the groundwater were more than two orders of magnitude lower than the lowest-observed-adverse-effect level (malformation effect) established for the parent compound. The acute values for the alga, invertebrate, and larval fish species in this study fell within the range

of acute values established in a prior study for several other freshwater species. As was the case for acute toxicity, the groundwater did not cause any short-term chronic toxicity to the green alga, cladoceran, or larval fish. The frog embryo test is an acute test; thus, chronic data were not obtained for the frog.

The concentrations of DIMP in the groundwater were found to be an order of magnitude or lower than the no-observed-adverse-effect levels (NOAEL) for short-term chronic exposure to the parent compound. The NOAELs for the alga (reduction in growth), invertebrate (reduction in neonate production), frog embryo (mortality), and larval fish (reduction in growth) were 711, 142, 398, and 142 mg/L, respectively. Based on the NOAELs, Superfund ecological screening-level risk assessment hazard quotients for the green alga, cladoceran, and larval fish would be 0.008, 0.042, and 0.042, respectively. The NOAEL for the frog embryo (mortality), which was established in an acute test, is 398 mg/L; the hazard quotient would be 0.015. A hazard quotient <1 indicates that a contaminant has a negligible potential for ecological impact. Thus, it was concluded that the apparent plume of DIMP migrating in the surficial aquifer from the Building E3640 Process Laboratory area towards Kings Creek should have minimal impact on the aquatic environment.

ACKNOWLEDGMENTS

We thank Mr. Tommy Shedd of the U.S. Army Center for Environmental Health Research (USACEHR), Fort Detrick, Frederick, Maryland, for his continual support and help throughout the study. Mr. Alan Rosencrance and Mr. Bill Dennis of USACEHR are acknowledged for conducting the explosive analyses. Thanks are extended to Mr. Steve Chapman, Ms. Rhonda Zuchowski, and Mr. Travis McCoun of The IT Group, Edgewood, Maryland, for taking the groundwater samples. We would also like to thank Mr. John Wrobel of the Environmental Conservation and Restoration Division of the Directorate of Safety, Health and Environment, Aberdeen Proving Ground, Maryland, and Ms. Allison O'Brien, an intern from the Oak Ridge Institute for Science and Education for their help during the study. We acknowledge USACEHR for supporting the study through U.S. Army Contract No. DAMD17-92-C-2066.

TABLE OF CONTENTS

	<u>Page</u>
FOREWORD	1
CERTIFICATION OF TECHNICAL DATA CONFORMITY	2
EXECUTIVE SUMMARY	3
ACKNOWLEDGMENTS	5
TABLE OF CONTENTS	6
LIST OF TABLES	8
LIST OF ABBREVIATIONS AND ACRONYMS	9
1. INTRODUCTION	10
2. MATERIALS AND METHODS	11
2.1 General	11
2.2 Acute Toxicity Tests	11
2.3 Short-term Chronic Toxicity Tests	12
2.3.1 Green Alga	12
2.3.2 Cladoceran	13
2.3.3 Fathead Minnow	13
2.4 Comprehensive Chemical Analyses	14
2.5 Test Endpoints and Data Analyses	14
3. RESULTS AND DISCUSSION	17
3.1 Groundwater Studies	17
3.2 Parent Compound Studies	18
3.2.1 Acute Toxicity	18
3.2.2 Chronic Toxicity	21
4. CONCLUSIONS	23
5. REFERENCES	24

TABLE OF CONTENTS (CONTINUED)

APPENDICES

APPENDIX 1	COMPREHENSIVE CHEMICAL, EXPLOSIVE, AND DIMP ANALYSES OF WELL NO. CCJ153A
APPENDIX 2	GREEN ALGAL 96-H GROWTH TEST CONDUCTED ON GROUNDWATER TAKEN FROM CANAL CREEK WELL NO. CCJ153A
APPENDIX 3	CLADOCERAN ACUTE AND 7-DAY SURVIVAL AND REPRODUCTION TEST CONDUCTED ON GROUNDWATER TAKEN FROM CANAL CREEK WELL NO. CCJ153A
APPENDIX 4	FATHEAD MINNOW ACUTE AND CHRONIC 7-DAY SURVIVAL AND GROWTH TEST CONDUCTED ON GROUNDWATER TAKEN FROM CANAL CREEK WELL NO. CCJ153A
APPENDIX 5	FROG EMBRYO TERATOGENESIS ASSAY - <i>XENOPUS</i> (FETAX) CONDUCTED ON GROUNDWATER TAKEN FROM CANAL CREEK WELL NO. CCJ153A
APPENDIX 6	GREEN ALGAL 96-H GROWTH TEST CONDUCTED WITH DIISOPROPYL METHYLPHOSPHONATE (DIMP)
APPENDIX 7	CLADOCERAN ACUTE AND 7-DAY SURVIVAL AND REPRODUCTION TEST CONDUCTED WITH DIISOPROPYL METHYLPHOSPHONATE (DIMP)
APPENDIX 8	FATHEAD MINNOW ACUTE AND CHRONIC 7-DAY SURVIVAL AND GROWTH TEST CONDUCTED WITH DIISOPROPYL METHYLPHOSPHONATE (DIMP)
APPENDIX 9	FROG EMBRYO TERATOGENESIS ASSAY (FETAX) - <i>XENOPUS</i> CONDUCTED WITH DIISOPROPYL METHYLPHOSPHONATE (DIMP)

LIST OF TABLES

Page

1.	SUMMARY OF THE DIMP TOXICITY DATA BASE FOR AQUATIC ORGANISMS	19
----	--	----

LIST OF ABBREVIATIONS AND ACRONYMS

°C	degrees centigrade
µg	microgram
µs/cm	microSiemens per centimeter
BCF	bioconcentration factor
CAS No.	Chemical Abstracts Service Registry Number
COR	Commanding Officer's Representative
cm	centimeter
d	day
DIMP	diisopropyl methylphosphonate
EC50	concentration of a material that causes an effect other than death to 50% of the test organisms
EPA	U.S. Environmental Protection Agency
FETAX	frog embryo teratogenesis assay - <i>Xenopus</i>
g	gram
GB	Sarin (isopropyl methylphosphonoflouridate)
h	hour
HCG	human chorionic gonadotropin
I.D.	inner diameter
I.U.	international unit
K _{ow}	octanol water partition coefficient
L	liter
LC50	concentration of a material lethal to 50% of the test organisms
LOAEL	lowest-observed-adverse-effect level
m	meter
mL	milliliter
mg/L	milligram per liter
mL/min	milliliter per minute
NOAEL	no-observed-adverse-effect level
PCBs	polychlorinated biphenyl compounds
PVC	polyvinyl chloride
RI/FS	Remedial Investigation/Feasibility Study
RO	reverse osmosis
rpm	revolutions per minute
TI	teratogenic index
UMD/WREC	University of Maryland Wye Research and Education Center
USACEHR	U.S. Army Center for Environmental Health Research

1. INTRODUCTION

Diisopropyl methylphosphonate (DIMP) was found in the subsurface soils and surficial groundwater in the vicinity of the Building E3640 Process Laboratory (Building E3650) during the 1994-1995 Remedial Investigation/Feasibility Study (RI/FS) for the Canal Creek Study Area of the Aberdeen Proving Ground - Edgewood Area (Jacobs Engineering Group Inc., 1995). Building E3640, which is located on the north side of Beach Point Road, was constructed in 1951 and 1952 and operated through 1978. Most of the work at the site involved the preparation of materials or evaluation of production processes. Research involving the disposal of chemical agents was also performed at Building E3640. DIMP was used as a precursor in the scale-up of bench syntheses of the chemical warfare agent GB (Sarin)(Battelle, 1997).

Most liquid wastes generated at Building E3640 were discharged to the chemical sewer system. Wastewater was collected from working bays in three sumps located below the floor on the north side of the building. Effluents from the sumps passed through a flow-through sump located northeast of the building and were discharged into an open ditch approximately 58 m (190 feet) northeast of the Building E3640 site. Wastewater was then carried northward in the open ditch to a branch of Kings Creek.

The presence of DIMP in 13 of 15 subsurface soil samples (vertical extent of contamination was evaluated to the water table) in the northeast corner of the site suggests that wastewater discharged to the sewer line contained DIMP (Jacobs Engineering Group Inc., 1995). DIMP was found in two surficial wells downgradient of a surficial groundwater divide where the groundwater flow is to the north toward Kings Creek. DIMP concentrations of 0.077 and 2.170 mg/L were found in the surficial wells CCJ152A and CCJ153A, respectively (Jacobs Engineering Group Inc., 1995).

The RI/FS concluded that an apparent plume of DIMP is migrating in the surficial aquifer from the Building E3640 area towards Kings Creek (Jacobs Engineering Group Inc., 1995). As a result, EPA recommended that additional information concerning the toxicity of DIMP to the aquatic organisms be obtained for the site. A review of the literature showed that acute toxicity data were available for several aquatic organisms; little data existed for chronic toxicity (Bentley et al., 1976; Van Voris et al., 1987). The current study was initiated to determine the acute and chronic toxicity of DIMP and the possible interactions of DIMP with other contaminants that may be present in the groundwater as it enters the aquatic environment. The toxicity of DIMP and its interaction with other contaminants in well CCJ153A was investigated as a worst case condition in the surficial aquifer north of the Building E3640 site. The toxicity of the parent compound was also determined to confirm the acute toxicity data in the literature and to provide chronic toxicity data which are not available.

2. MATERIALS AND METHODS

2.1 General

Groundwater was withdrawn from well CCJ153A on March 16, 18, and 20, 1998 by personnel from The IT Group (Edgewood, Maryland) following approved standard operation procedures for the Aberdeen Proving Ground Installation Restoration Program. The well is 3.6 m deep (11.0 feet) and has a screened interval of 1.8-3.6 m (6.0-11.0 feet) (Jacobs Engineering Group Inc., 1995). The height of the well stickup is 0.8 m (2.5 feet). The actual depth to the water ranged from 1.0-1.2 m (3.2-3.8 feet) during the three sampling periods. The well was purged each sampling day at a rate of 600 mL/min until pH, temperature, dissolved oxygen, redox potential, and conductivity stabilized within 10%. Sufficient groundwater was taken at 600 mL/min for chemical analyses (see below) and test solution for the bioassays. The peristaltic pump battery failed during sampling on March 16, 1998; thus, sampling was completed via a disposable bailer. All groundwater samples were placed on ice during collection in the field and subsequent delivery for chemical analyses and bioassays.

DIMP (CAS No. 1445-75-6) used in the parent compound assays was obtained from USACEHR. The DIMP was >99% pure. The stock solution used in the bioassays was prepared from the pure solution and quantified by GPL Laboratories, LLLP, Gaithersburg, Maryland. Nominal test concentrations were prepared from the stock solution and corrected based on the measured concentration of the stock solution.

The acute and chronic toxicity of DIMP and the possible interactions of DIMP with other contaminants that may be present in the groundwater and the parent compound were both evaluated using an array of four biomonitoring systems which included a number of endpoints. The biomonitoring assays covered several levels of biological complexity to maximize the predictability of DIMP toxicity to aquatic organisms. The following toxicity tests were conducted: 96-h algal (*Selenastrum capricornutum*) growth test; 7-d cladoceran (*Ceriodaphnia dubia*) survival and reproduction test; and 7-d fathead minnow (*Pimephales promelas*) survival and growth test. In addition, survival and developmental toxicity were determined by the 96-h frog embryo teratogenesis assay-*Xenopus* (FETAX) using the African clawed frog, *Xenopus laevis*. The experimental procedures for each assay are briefly described below.

2.2 Acute Toxicity Tests

Acute toxicity values were calculated where possible for the cladoceran and fathead minnow from the data obtained during the short-term chronic tests described in Section 2.3. Forty-eight-h LC50s and 96-h LC50s were determined where possible for the cladoceran and fathead minnow, respectively. With regard to the green alga, EPA's Toxic Substance Control Act office considers the 96-h test to be an acute test for

determining the toxicity of single compounds (U.S. EPA, 1985 and 1986a). EPA's Office of Research and Development considers the 96-h algal test for growth to be a short-term chronic test for determining the toxicity of effluents (Horning and Weber, 1985; Weber et al., 1989; Lewis et al., 1994) as do other investigators for evaluating single chemicals (for ex., see Hughes et al., 1988 and Suter, 1993). We analyzed the data as chronic data because the short-term chronic test method was used (Section 2.3). The 96-h EC50 for cell density (Section 2.3.1) was also used as an acute value for comparative purposes with acute data in the literature.

Developmental toxicity tests were conducted on the groundwater and parent compound using the frog embryo teratogenesis assay - *Xenopus* (FETAX). The assay is a 96-h quantitative developmental assay used to screen for developmental toxicants in aquatic media. The assays were conducted using the static renewal (solutions renewed every 24 h) test method Designation E 1439-91 of the American Society for Testing and Materials (ASTM, 1998). Embryo lethality (96-h LC50) and malformations (96-h EC50) were determined when >50% mortality and/or malformations occurred; growth retardation was not evaluated. The identification and interpretation of malformations in the embryos at 96 h were made via the atlas of Bantle et al. (1991).

Embryos between normal stage 8 blastulae and normal stage 11 gastrulae were obtained from *X. laevis* breeding colonies at the UMD/WREC as described below. The embryos were de-jellied in a 2% L-cysteine solution (2 g of L-cysteine per 98 mL of FETAX solution). Once de-jellied, the embryos were rinsed and re-suspended in FETAX solution (ASTM, 1998). The embryos were tested in glass petri dishes containing 10 mL of solution. Two replicates of 25 embryos/replicate were used for each test treatment. The tests were conducted at $24 \pm 0.2^\circ\text{C}$ under a 16-h light: 8-h dark photoperiod (fluorescent lights; ~75 foot candles at the surface of the test medium) in a constant temperature environmental chamber.

The UMD/WREC *X. laevis* adult colony was maintained in flow-through (~4 replacement volumes per day) circular polyethylene aquaria (0.91 m I.D. x 0.36 m high) with a water depth of 10 cm. Each aquarium contained a maximum of 10 adults. UMD/WREC non-chlorinated deep well water (water quality given in Section 2.3.3) held at $23.5 \pm 0.5^\circ\text{C}$ served as the culture medium. All frogs were fed every 5-6 d with commercial beef liver supplemented with liquid vitamins (PolyViSol™; Mead-Johnson Nutritional, Evansville, Indiana). The colony was held under a photoperiod of 16 h light: 8 h dark. Mating pairs were bred in the dark in $23.5 \pm 0.5^\circ\text{C}$ UMD/WREC non-chlorinated water at ~70 d intervals by injecting 400 and 800 I.U. of human chorionic gonadotropin (HCG) in the dorsal lymph sac of the males and females, respectively. Amplexus occurred 4-6 h after injecting HCG; egg deposition occurred 9-12 h following HCG injection.

2.3 Short-term Chronic Toxicity Tests

2.3.1 Green Alga

The short-term chronic toxicity of the groundwater and parent compound to the green alga (*S. capricornutum*) was determined by the EPA procedures given in Lewis et al. (1994). Stock algal cultures were reared in 2.5 L Pyrex® culture flasks containing 1 L of sterilized algal assay medium. Cultures were maintained in a constant temperature incubator under constant cool-white fluorescent lights (~300 foot candles) at a temperature of $25 \pm 0.2^\circ\text{C}$ on a shaker table oscillating at 100 ± 10 rpm. Log growth cells were used to start all tests.

Algal test solutions were prepared by dilution of the groundwater with filtered sterilized assay media or the addition of stock DIMP into the algal assay media. Test solutions (100 mL total volume) were dispensed into 250 mL Delong flasks and inoculated with *S. capricornutum* cells to achieve a density of $\sim 1 \times 10^4$ cells/mL. Triplicates were prepared for each treatment. The flasks were placed on a shaker table in an incubator set at the culturing conditions described above. Growth measurements (cell density) were made from all replicates in each treatment at 96 h. Algal cell density was determined from a 1 mL sample with a Model ZBI Coulter Counter (Coulter Electronics, Inc., Hialeah, Florida). The instrument was calibrated with each use via hemocytometer counts. Test solutions were not renewed during the 96-h studies.

2.3.2 Cladoceran

The chronic toxicity of the groundwater and parent compound to *C. dubia* was determined by the EPA static renewal method (solutions renewed daily) given in Lewis et al. (1994). The cladoceran was cultured at $25 \pm 1^\circ\text{C}$ in 600 mL glass beakers filled with 400 mL of 20% Perrier:80% reverse osmosis water. The diet consisted of a mixture of Cerophyl® (Cerophyl Laboratories, Inc., Kansas City, Missouri) and the green alga, *S. capricornutum*, added to the cladoceran culture to achieve final concentrations of 120 µg Cerophyl®/mL and $\sim 6.7 \times 10^5$ *S. capricornutum* cells/mL.

All neonates used in the 7-d survival and reproduction tests were produced by cladocerans in culture that had released at least three broods. The initial age of the neonates in each test was <4 h old. The tests were conducted in 50 mL glass beakers containing 25 mL of test solution. All tests were conducted in an environmental chamber at $25 \pm 1^\circ\text{C}$ under a 16-h light:8-h dark photoperiod (fluorescent lights; 60-85 foot candles at the surface of the culture vessels). All test organisms were fed daily as described above at each 24-h renewal. Routine water quality was taken at the beginning and end of each 24-h renewal.

2.3.3 Fathead Minnow

The toxicity of the groundwater and parent compound to fathead minnows (*P. promelas*) was determined by the EPA static renewal method (solutions renewed daily) given in Lewis et al. (1994). All larvae used in the 7-d survival and growth tests were <24 h old at the start of the test. The tests were conducted in 600 mL glass beakers

containing 400 mL of test solution. The dilution water was a 20% Perrier:80% reverse osmosis water. All test organisms were fed brine shrimp (*Artemia* sp.) nauplii <24 h old daily at each 24-h renewal. All tests were conducted at $25 \pm 1^\circ\text{C}$ under a 16-h light:8-h dark photoperiod (fluorescent lights; 60-85 foot candles). Routine water chemistry was performed at the beginning and end of each renewal. Dry weight was determined by drying at 100°C for a minimum of 12 h.

Fathead minnow larvae were obtained from the UMD/WREC in-house culture maintained at $25 \pm 1^\circ\text{C}$ in non-chlorinated well water (mean dissolved oxygen = 8.1 mg/L; conductivity = 159 $\mu\text{S}/\text{cm}$; alkalinity = 54 mg/L as CaCO_3 ; hardness = 52 mg/L as CaCO_3 ; pH ranged from 7.1 to 8.0). The UMD/WREC culture procedures were similar to those recommended by Peltier and Weber (1985).

Spawning fish were cultured in 38 L (10 gallon) flow-through glass aquaria supplied with UMD/WREC well water ($25 \pm 1^\circ\text{C}$) which was continually filtered and sterilized via ultraviolet light. The spawning adults were fed a diet of TetraMin® Staple Food (Ramfab Aquarium Products Co., Oak Ridge, Tennessee) twice daily. Excess food was removed daily. Eight sets of spawning fathead minnows were maintained in the culture tanks at a ratio of 1 male:4 females. Replacement spawners were rotated at approximately three-month intervals. Fathead minnow embryos were collected on spawning substrates (10 cm I.D. x 20 cm long PVC pipe sections cut longitudinally in equal portions) and transferred to 19 L aquaria at $25 \pm 1^\circ\text{C}$ in UMD/WREC well water for hatching. All stages of the fish were reared under a 16-h light:8-h dark photoperiod (fluorescent lights; 60-85 foot candles).

2.4 Comprehensive Chemical Analyses

Comprehensive chemical, explosives, and DIMP analyses were performed on each groundwater sample taken March 16, 18, and 20, 1998. The comprehensive chemical analyses included general water chemistry, metals, volatile organics, base neutrals, acid compounds, pesticides/PCBs, and herbicides. The explosive analyses included both nitroaromatic and nitramine explosives. The elements and/or compounds analyzed in each group, detection limits, analytical methods, etc., are given in Appendix 1.

All field samples were placed in appropriate containers provided by the vendors for the various analyses. The containers were placed on ice and delivered to the vendors by the afternoon the samples were taken for the analyses. The comprehensive chemical analyses were performed by Gascoyne Laboratories, Inc., Baltimore, Maryland, by the methods given in Appendix 1. The explosive samples were analyzed by USACEHR (USACEHR, 1993). The DIMP analyses were analyzed by GPL Laboratories, LLLP, Gaithersburg, Maryland (GPL Laboratories, LLLP, 1998).

2.5 Test Endpoints and Data Analyses

The test endpoint for the effects of groundwater and the parent compound for the green alga after 96 h of exposure was growth, measured as cell density (cells/mL). The endpoints for the 7-d survival and reproduction tests with the cladoceran were survival and young production. The test endpoints for the fathead minnow 7-d survival and growth tests were survival and growth. The endpoints for the frog test were embryo survival and malformations after 96 h of exposure.

The 96-h EC50 for green algal growth was estimated by using the "inhibition proportion" technique recommended by Horning and Weber (1985). The technique uses quantal analyses (e.g., probit or Trimmed Spearman-Kärber methods) to estimate EC50s and their 95% fiducial or confidence limits. Since the assumptions of the quantal analysis are not met in the classical sense because of the very nature of the growth data, the count data at each treatment were averaged and subsequently converted to "inhibition proportions" using the following formula:

$$I = C - T / C (100)$$

where: C = the mean growth of the controls

T = the mean growth at a given treatment

The 96-h EC50 for algal growth in the parent compound assay was estimated by the probit statistic (Stephan, 1978).

The 48-h and 7-d LC50s (and their 95% confidence limits) with cladocerans and 96-h and 7-d LC50s with fathead minnows were determined in the parent compound tests by the Trimmed Spearman-Kärber method (U.S. EPA, 1993). The 96-h LC50 and 96-h EC50 and their 95% confidence limits for embryo survival and malformations in the FETAX parent compound assay were also determined by the Trimmed Spearman-Kärber method (U.S. EPA, 1993a).

The no-observed-adverse-effect level (NOAEL) and lowest-observed-adverse-effect level (LOAEL) for green algal growth (cells/mL) exposed to the parent compound were determined by Dunnett's test. Dunnett's test consists of an analysis of variance (ANOVA) to determine the error term, which is then used in a multiple comparison test for comparing each of the treatment means with the control mean. The assumptions upon which the use of Dunnett's test are contingent are that the observations within treatments are independent and normally distributed, with homogeneity of variance. The Shapiro-Wilk's test for normality and Bartlett's test for homogeneity of variances were performed before the Dunnett's test was used. The above statistical tests were performed using Toxstat (WEST and Gulley, 1994) at a minimum probability level of 0.05.

The NOAEL and LOAEL for the adult cladoceran raw survival data in the parent compound assay were analyzed by Fisher's Exact test. An arc-sine square root transformation was made on the fathead minnow percent survival raw data before

further data analyses were performed to estimate the NOAEL and LOAEL. The raw data for cladoceran neonate production and fathead minnow larval growth were not transformed before the NOAEL and LOAEL statistics were performed. With the exception of the cladoceran survival data, all data were then subjected to a Shapiro-Wilk's test for normality and Bartlett's test for homogeneity of variance. When the data sets met the assumptions of normality and homogeneity of variance, Dunnett's parametric statistic was used. When a data set failed to meet the assumptions of normality or homogeneity of variance, Steel's Many-One Rank nonparametric statistic was used. The statistical tests were performed using Toxstat (WEST and Gulley, 1994). A minimum probability level of 0.05 was used for all tests.

The NOAEL and LOAEL for the FETAX percent embryo survival and percent embryo malformation parent compound data were estimated by Dunnett's method following arc sine square transformation with the following exception. The data were assumed to be normally distributed, with homogeneity of variance after arc-sine square root transformation. The transformed data could not be tested for normality and homogeneity of variance because two replicates were used in the assay as required by the ASTM test protocol (ASTM, 1998). Estimates of the NOAEL and LOAEL are not recommended endpoints in the FETAX protocol; however, we estimated the two metrics in order to compare the frog NOAELs and LOAELs with the other three test species. The statistical tests were performed using Toxstat (WEST and Gulley, 1994). A minimum probability level of 0.05 was used.

3. RESULTS AND DISCUSSION

3.1 Groundwater Studies

The concentrations of DIMP in the surficial groundwater samples taken from well CCJ153A on March 16, 18, and 20, 1998, were 6.05, 5.09, and 4.72 mg/L, respectively (Appendix 1). A DIMP concentration of 2.17 mg/L was reported for the well in the RI/FS (Jacobs Engineering Group Inc., 1995). The concentration of the priority pollutant heavy metal chromium was 4 µg/L at each sample period. A concentration of 5 µg/L total copper was found on March 16, 1998; total copper was below the detection limit of 5 µg/L on March 18 and 20, 1998. Aluminum, barium, and manganese concentrations ranged from 22-74, 60-67, and 970-1,000 µg/L, respectively. The only priority pollutant volatile organic found above the detection limits given in Appendix 1 was vinyl chloride (29-32 µg/L). No base neutrals, acid extractables, organophosphorus pesticides, or chlorinated pesticides and herbicides were found in the groundwater above the detection limits given in Appendix 1. No nitroaromatic or nitramine explosives above a detection limit of 50 µg/L were present (Appendix 1). The general water quality of the groundwater (pH, hardness, total organic carbon, etc.) is also given in Appendix 1. The original data sheets and quality control data for all chemical analyses are archived at USACEHR.

The groundwater bioassay data for the alga, cladoceran, fathead minnow, and frog are given in Appendices 2-5, respectively. The groundwater from well CCJ153A was not acutely or chronically toxic to the alga, cladoceran, or fathead minnow. Specifically, the groundwater did not affect the growth (cell density) of algae after 96 h of exposure (Appendix 2; Tables A2-2 and A2-3). No acute or short-term chronic effects on the survival of adult cladocerans occurred after 48 h and 7 d of exposure, respectively (Appendix 3; Tables A3-2 and A3-3). The groundwater had no effect on cladoceran neonate production after 7 d of exposure (Appendix 3; Tables A3-3 and A3-4). The groundwater had no 48-h acute or 7-d short-term chronic effect on the survival of fathead minnow larvae (Appendix 4; Tables A4-2, A4-3, and A4-4). Likewise, the groundwater had no effect on fathead minnow larval growth after 7 d of exposure (Appendix 4; Tables A4-3 and A4-5).

Groundwater from well CCJ153A had no effect on frog embryo survival after 96 h of exposure (Appendix 5; Tables A5-2 and A5-3). A statistically significant ($\alpha = 0.05$) effect was found for embryo malformations (Appendix 5; Tables A5-2, A5-4, and A5-5); however, it appears that the effect was statistical error (i.e., 1 in 20 chance of accepting the null hypothesis that all groups are equal). The concentration of DIMP in 100% groundwater averaged 5.3 mg/L during the bioassay. As shown in Section 3.2, the concentration of DIMP which caused a malformation effect (LOAEL) was 569 mg/L which is more than two orders of magnitude higher than the concentration in the groundwater during the test. The concentration of priority pollutants present in well

CCJ153A were below the concentrations that are toxic to freshwater aquatic organisms (see below).

The fact that the surficial groundwater in well CCJ153A was not toxic to the test species is not surprising when one considers the low concentrations of DIMP and priority pollutants present in the groundwater. As discussed in Section 3.2, the toxicity of DIMP is an order of magnitude or higher than the concentrations present in the surficial groundwater. The concentration of total chromium present in the groundwater (4 µg/L) was lower than the EPA freshwater chromium acute (16 µg/L) and chronic (11 µg/L) numerical water quality criteria for chromium (VI) which is the most toxic form of chromium (U.S. EPA, 1984a). The concentration of total copper (5 µg/L) in one of the three groundwater samples (March 16, 1998) was equal to EPA's freshwater acute copper criteria of 5 µg/L and above the EPA chronic criteria of 4 µg/L when the EPA criteria were adjusted to a hardness of 28 mg/L as CaCO₂ which was the average hardness of the groundwater (U.S. EPA, 1984b). The concentration of total copper was below the detection limit of 5 µg/L on two of the three sample days (Appendix 1). EPA points out in their ambient water quality criteria document for copper that the total copper criteria may be overly protective (U.S. EPA, 1984b). It is well established that the divalent cation of copper is the most toxic oxidation state of copper to freshwater organisms (Lee, 1973). Aluminum concentrations in the groundwater ranged from 22-74 µg/L. The ambient water quality criteria for aluminum are 750 and 87 µg/L for the acute and chronic criteria, respectively, in the pH range of 6.5-9.0 (U.S. EPA, 1988). EPA acute and/or chronic numerical water quality criteria are not available for barium, manganese, and vinyl chloride which were also present in the groundwater (U.S. EPA, 1986b).

3.2 Parent Compound Studies

3.2.1 Acute Toxicity

The DIMP parent compound test data for the alga are given in Appendix 6 and summarized in Table 1. The acute 96-h EC50 (inhibition of growth) for *S. capricornutum* was 3,185 mg/L. A 96-h EC50 of 2,623 mg/L was established by Bentley et al. (1976) for the same algal species. Van Voris et al. (1987), found that a concentration of 500 mg/L, which was the highest concentration studied, had no effect on *S. capricornutum* during their logarithmic growth phase (4-7 days). Bentley et al. (1976) also established 96-h EC50s (inhibition of growth) of 2,234, 6,107, and 2,345 mg/L DIMP for the freshwater algae *Microcystis aeruginosa*, *Anabeana flos-aquae*, and *Navicula pelliculosa*, respectively (Table 1). Van Voris et al. (1987) found that a concentration of 500 mg/L, which was the highest concentration studied, had no effect on the freshwater alga *Chlorella pyrenoidosa* during their logarithmic growth phase (4-7 days).

**TABLE 1. SUMMARY OF THE DIMP TOXICITY DATA BASE
FOR AQUATIC ORGANISMS^a**

Species	Endpoint	Current Study	Other Studies
ACUTE TOXICITY			
Algae:			
<i>Selenastrum capricornutum</i>	96-h EC50 ^b	3,185	2,623 ^c >500 ^d
<i>Microcystis aeruginosa</i>	96-h EC50 ^b		2,234 ^c
<i>Anabeana flos-aquae</i>	96-h EC50 ^b		6,107 ^c
<i>Navicula pelliculosa</i>	96-h EC50 ^b		2,345 ^c
<i>Chlorella pyrenoidosa</i>	96-h EC50 ^b		>500 ^d
Invertebrates:			
<i>Ceriodaphnia dubia</i>	48-h LC50	610	
<i>Daphnia magna</i>	48-h LC50		267 ^c
<i>Gammarus fasciatus</i>	48-h LC50		494 ^c
<i>Asellus militaris</i>	48-h LC50		2,160 ^c
<i>Chironomous tentans</i>	48-h LC50		1,720 ^c
Amphibian:			
<i>Xenopus laevis</i>	96-h LC50	1,543	
	96-h EC50 ^e	1,225	
	NOAEL ^f	398	
	LOAEL ^f	569	
Fish:			
<i>Pimephales promelas</i>	96-h LC50	604	479 ^c
<i>Ictalurus punctatus</i>	96-h LC50		285 ^c
<i>Lepomis macrochirus</i>	96-h LC50		406 ^c
<i>Oncorhynchus mykiss</i>	96-h LC50		631 ^c

TABLE 1. (CONTINUED)

Species	Endpoint	Current Study	Other Studies
CHRONIC TOXICITY			
Alga:			
<i>Selenastrum capricornutum</i>	NOAEL ^b	711	
	LOAEL ^b	1,423	
Invertebrate:			
<i>Ceriodaphnia dubia</i>	7-d LC50	375	
	NOAEL ^g	142	
	LOAEL ^g	285	
Fish:			
<i>Pimephales promelas</i>	7-d LC50	381	
	NOAEL ^h	142	
	LOAEL ^h	285	
<i>Lepomis macrochirus</i>	14-d Bioconcentration		>167 ⁱ

^a All toxicity values are given as mg/L DIMP.

^b Test endpoint- reduction in growth (cell density).

^c Bentley et al. (1976).

^d 500 mg/L DIMP highest concentration studied (Van Voris et al., 1987).

^e Test endpoint- increase in malformations.

^f Test endpoint- mortality.

^g Test endpoint- reduction in neonate production.

^h Test endpoint- reduction in growth.

ⁱ 167 mg/L DIMP highest concentration studied; no apparent stress or bioconcentration of DIMP occurred during a 14-d exposure (Bentley et al., 1976).

The 48-h LC50 for the invertebrate *C. dubia* was 610 mg/L (Appendix 7). A 48-h LC50 of 267 mg/L was found by Bentley et al. (1976) for *Daphnia magna* another species in the same family as *C. dubia* (Table 1). As shown in Table 1, Bentley et al. (1976) also established 48-h LC50s for the scud (*Gammarus fasciatus*), sowbug (*Asellus militaris*), and midge (*Chironomus tentans*) which were 494, 2,160, and 1,720 mg/L DIMP, respectively.

The acute toxicity (96-h LC50) of DIMP to the larval fathead minnow (*P. promelas*) was 604 mg/L in the current study (Appendix 8). Bentley et al. (1976) obtained a 96-h

LC50 of 479 mg/L for the same fish (Table 1). As summarized in Table 1, the 96-h LC50s found in the Bentley et al. (1976) study for the channel catfish (*Ictalurus punctatus*), bluegill (*Lepomis macrochirus*), and rainbow trout (*Oncorhynchus mykiss*) were 285, 406, and 631 mg/L, respectively.

The 96-h LC50 for frog embryos exposed to DIMP was 1,543 mg/L (Appendix 9). The 96-h NOAEL and LOAEL for mortality were 398 and 569 mg/L, respectively. The 96-h EC50 for malformations was 1,225 mg/L. The predominate malformations were multiple edema (50% of the embryos) and gut coiling (28%) followed by severe (9%) and notochord (9%); cardiac, face, and eye each accounted for ~1% of the malformations (Appendix 9; Table A9-6). A NOAEL and LOAEL for malformations could not be determined because significant mortality occurred at exposure concentrations of 569 mg/L and above (Appendix 9; Table A9-5). The teratogenic index (TI), which by definition is the 96-h LC50 divided by the 96-h EC50 (malformations), provides an estimate of the teratogenic risk associated with a material (Dumont et al., 1983). TI values of 1.5 to 2.0 indicate that a material may be a potential teratogen. Materials with TI values >2.0 should be considered for further teratogenicity testing. The TI in the current study was ~1.3; thus, a low potential exists that DIMP is a developmental hazard.

3.2.2 Chronic Toxicity

The chronic toxicity of DIMP to the green alga, cladoceran, and fathead minnow are summarized in Table 1. The green alga (*S. capricornutum*) NOAEL and LOAEL (reduction in cell density) were 711 and 1,423 mg/L DIMP, respectively (Appendix 6). The 7-d LC50 for the cladoceran exposed to DIMP was 375 mg/L (Appendix 7). The cladoceran NOAEL and LOAEL (reduction in neonate production) were 142 and 285 mg/L, respectively (Table 1). The larval fathead minnow 7-d LC50 was 381 mg/L DIMP (Appendix 8). The NOAEL and LOAEL (reduction in larval growth) were 142 and 285 mg/L, respectively.

With the exception of a 14-d bioconcentration study, no other chronic DIMP data were found in the literature for aquatic organisms. Bentley et al. (1976) conducted a bioconcentration study with bluegill exposed to 167 mg/L ¹⁴C-DIMP for 14 d followed by a 7-d depuration phase. According to the authors, the bluegill appeared normal, fed readily, and generally showed no signs of stress during the study. No bioconcentration of DIMP occurred in the study. Bioconcentration of DIMP would not be expected when one considers that the log K_{ow} for DIMP is 1.03 (Krikorian et al., 1987). Bioconcentration of a material up to 100-fold above background (bioconcentration factor or BCF = 100) normally does not occur until log K_{ow} = 3 (U.S. EPA, 1991).

In summary, the concentrations of DIMP in the groundwater were found to be an order of magnitude or lower than the NOAELs for short-term chronic exposure to the parent compound. The NOAELs for the alga (reduction in growth), invertebrate (reduction in neonate production), frog embryo (mortality), and larval fish (reduction in growth) were 711, 142, 398, and 142 mg/L, respectively. Based on the NOAELs, Superfund screening-level risk assessment hazard quotients for the green alga, cladoceran, frog embryo, and larval

fish would be 0.008, 0.042, 0.015, and 0.042, respectively (Burton, 1999). A hazard quotient <1 indicates that a contaminant has a negligible potential for ecological impact (U.S. EPA, 1997). Thus, the apparent plume of DIMP migrating in the surficial aquifer from the Building E3640 Process Laboratory area towards Kings Creek should have minimal impact on the aquatic environment.

4. CONCLUSIONS

The concentrations of DIMP in the surficial groundwater samples taken from well CCJ153A on March 16, 18, and 20, 1998 at the Building E3640 Process Laboratory site, were 6.05, 5.09, and 4.72 mg/L, respectively. Low concentrations of several priority pollutant heavy metals (aluminum, barium, chromium, copper, and manganese) and one volatile organic (vinyl chloride) were also present in one or more of the three groundwater samples. No base neutrals, acid extractables, organophosphorus pesticides, or chlorinated pesticides and herbicides were found in the groundwater above the detection limits for drinking water. No nitroaromatic or nitramine explosives above a detection limit of 50 µg/L were present.

The groundwater was not acutely toxic to a freshwater green alga (*Selenastrum capricornutum*), invertebrate (*Ceriodaphnia dubia*), or larval fish (*Pimephales promelas*). The groundwater was not acutely toxic to frog embryos (*Xenopus laevis*) after 96 h of exposure. A statistically significant ($\alpha = 0.05$) effect was found for frog embryo malformations; however, the effect was judged to be statistical error because the concentrations in the groundwater were more than two orders of magnitude lower than the LOAEL (malformation effect) established for the parent compound. The acute values for the alga, invertebrate, and larval fish species in this study fell within the range of acute values established in a prior study for several other freshwater species. As was the case for acute toxicity, the groundwater did not cause any chronic toxicity to the green alga, cladoceran, or larval fish. The frog test is an acute test; thus, chronic data were not obtained for the frog.

The concentrations of DIMP in the groundwater were found to be more than an order of magnitude lower than the NOAELs for short-term chronic exposure to the parent compound. The NOAELs for the alga (reduction in growth), invertebrate (reduction in neonate production), frog embryo (mortality), and larval fish (reduction in growth) were 711, 142, 398, and 142 mg/L, respectively. Based on the NOAELs, Superfund ecological screening-level risk assessment hazard quotients for the green alga, cladoceran, and larval fish would be 0.008, 0.042, and 0.042, respectively (Burton, 1999). The NOAEL for the frog embryo (mortality), which was established in an acute test, is 398 mg/L; the hazard quotient would be 0.015 (Burton, 1999). A hazard quotient <1 indicates that a contaminant has a negligible potential for ecological impact (U.S. EPA, 1997). Thus, the apparent plume of DIMP migrating in the surficial aquifer from the Building E3640 Process Laboratory area towards Kings Creek should have minimal impact on the aquatic environment.

5. REFERENCES

- APHA et al. (American Public Health Association, American Water Works Association, and Water Pollution Control Federation). 1995. Standard methods for the examination of water and wastewater, 19th ed. Amer. Public Hlth. Assoc., Washington, DC.
- ASTM (American Society for Testing and Materials). 1998. Standard guide for conducting the frog embryo teratogenesis assay-Xenopus (FETAX). ASTM Designation E 1439-91. Pages 826-836 in: 1998 Annual book of ASTM standards, Vol. 11.05. Amer. Soc. Testing Materials, Philadelphia, PA.
- Bantle, J.A., J.N. Dumont, R.A. Finch, and G. Linder. 1991. Atlas of abnormalities a guide for the performance of FETAX. Oklahoma State Univ., Stillwater, OK.
- Battelle. 1997. Chemistry and environmental fate of diisopropyl methylphosphonate (DIMP). Rep. No. 2144-A-3. Battelle Pacific Northwest Laboratory Edgewood Operations, Aberdeen Proving Ground, MD.
- Bentley, R.E., G.A. LeBlanc, T.A. Hollister, and B.H. Sleight, III. 1976. Acute toxicity of diisopropyl methylphosphonate and dicyclopentadiene to aquatic organisms. AD A037750. National Tech. Information Service, Springfield, VA.
- Burton, D.T. 1999. Screening-level ecological risk assessment of diisopropyl methylphosphonate (DIMP) at the Building E3640 Process Laboratory, Edgewood Area - Aberdeen Proving Ground, Maryland. Draft Rep. University of Maryland at College Park, Queenstown, MD.
- Dumont, J., T. Schultz, M. Buchanan, and G. Kao. 1983. Frog embryo teratogenesis assay-Xenopus (FETAX) - A short-term assay applicable to complex environmental mixtures. Pages 393-405 in: Waters, Sandhu, S.S., J. Lewtas, L. Claxton, N. Chernoff, and S. Nesnow, eds. Short-term bioassays in the analysis of complex environmental mixtures III, Plenum, New York, NY.
- GPL Laboratories, LLLP. 1998. GP Environmental Services method T8 GC-FPD. GPL Laboratories, LLLP, Gaithersburg, MD.
- Horning, W.B. and C.I. Weber (eds.). 1985. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. EPA/600/4-85/014. U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH.

- Hughes, J.S., M.M. Alexander, and K. Balu. 1988. An evaluation of appropriate expressions of toxicity in aquatic plant bioassays as demonstrated by the effects of atrazine on algae and duckweed. Pages 531-547 *in*: Adams, W.J., G.A. Chapman, and W.G. Landis, eds. Aquatic toxicology and environmental fate, Vol. 10. ASTM STP 971. Amer. Soc. Testing Materials, Philadelphia, PA.
- Jacobs Engineering Group Inc. 1995. Remedial investigation progress report Canal Creek Study Area Aberdeen Proving Ground - Edgewood Area, Maryland. EMO-35E35610-B7-06357. Jacobs Engineering Group Inc., Arlington, VA.
- Krikorian, S.E., T.A. Chorn, and J.W. King. 1987. Determination of O/W partition coefficients of certain organophosphorus compounds using high performance liquid chromatography. *Quat. Struct. Act. Relat.* 6:65-70.
- Lee, G.F. 1973. Chemical aspects of bioassay techniques for establishing water quality criteria. *Water Res.* 7:1525-1546.
- Lewis, P.A., D.J. Klemm, J.M. Lazorchak, T.J. Norberg-King, W.H. Peltier, and M.A. Heber. 1994. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. EPA/600/4-91/002. U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH.
- Peltier, W.H. and C.I. Weber. 1985. Methods for measuring the acute toxicity of effluents to freshwater and marine organisms, 3rd ed. EPA/600/4-85/013. U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH.
- Stephan, C.E. 1978. EPA statistics program, version 1.4. U.S. Environmental Protection Agency, Environmental Research Laboratory, Duluth, MN.
- Suter, G.W., II. 1993. Organism-level effects. Pages 175-246 *in*: Suter, G.W., II, ed. Ecological risk assessment. Lewis Publ., Ann Arbor, MI.
- USACEHR (U.S. Army Center for Environmental Health Research). 1993. SOP for the determination of HMX, RDX, TNB, Tetryl, TNT, 2,6-DNT, and 2,4-DNT in water by high performance liquid chromatography (HPLC). No SOP Number. U.S. Army Center for Environmental Health Research, Ft. Detrick, Frederick, MD.
- U.S. EPA. 1984a. Ambient water quality criteria for chromium - 1984. EPA 440/5-84-029. U.S. Environmental Protection Agency, Washington, DC.
- U.S. EPA. 1984b. Ambient water quality criteria for copper - 1984. EPA 440/5-84-031. U.S. Environmental Protection Agency, Washington, DC.

- U.S. EPA. 1985. Toxic substances control act test guidelines; final rules. Federal Register 50:39252-39516.
- U.S. EPA. 1986a. Revision of toxic substances control act test guidelines. Federal Register 51:1522-1543.
- U.S. EPA. 1986b. Quality criteria for water 1986. EPA/5-86-001. U.S. Environmental Protection Agency, Washington, DC.
- U.S. EPA. 1988. Ambient water quality criteria for aluminum - 1988. EPA 440/5-86-008. U.S. Environmental Protection Agency, Washington, DC.
- U.S. EPA. 1991. Assessment and control of bioconcentratable contaminants in surface waters. 1991 Draft Report. U.S. Environmental Protection Agency, Washington, DC.
- U.S. EPA. 1993. Trimmed Spearman-Kärber program, v. 1.5. U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH.
- U.S. EPA. 1997. Ecological risk assessment guidance for superfund: Process for designing and conducting ecological risk assessments. EPA540-R-97-006. U.S. Environmental Protection Agency, Washington, DC.
- Van Voris, P., D.A. Cataldo, M.W. Ligothe, J.K. Fredrickson, S-m. W. Li, E.A. Creclius, J.T. Hardy, R.J. Fellows, and R.S. Wentzel. 1987. Acute environmental toxicity and persistence of selected chemical agent simulants: Diisopropyl fluorophosphate (DFP) and diisopropyl methylphosphonate (DIMP). CRDEC-CR-87071. Chemical Research Development Engineering Center, Aberdeen Proving Ground, MD.
- Weber, C.I., W.H. Peltier, T.J. Norberg-King, W.B. Horning, II, F.A. Kessler, J.R. Menkedick, T.W. Neihsel, P.A. Lewis, D.J. Klemm, Q.H. Pickering, E.L. Robinson, J.M. Lazorchak, L.J. Wymer, and R.W. Freyberg. 1989. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms, 2nd ed. EPA/600/4-89/001. U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH.
- WEST (Western Ecosystem Technology, Inc.) and Gulley, D. D. 1994. Toxstat, v. 3.4. Western Ecosystem Technology, Inc., Cheyenne, WY.

APPENDIX 1

**COMPREHENSIVE CHEMICAL, EXPLOSIVE, AND DIMP ANALYSES
OF WELL NO. CCJ153A**

TABLE A-1. COMPREHENSIVE CHEMICAL, EXPLOSIVE, AND DIMP ANALYSES OF WELL NO. CCJ153A -
GENERAL WATER QUALITY^a

Analyte	EPA Method	Detection Limits	March 16, 1998	March 18, 1998	March 20, 1998
Acid Soluble Sulfide (as S)	9030B	1	<1	<1	<1
Alkalinity (CaCO ₃)	310.1	1	28	29	29
Ammonia (as N)	^b	1	1	2	2
Bromide (Br)	9056	0.05	0.11	0.11	0.13
Chloride (Cl)	9056	0.5	18	18	18
Fluoride (as F)	9056	0.05	0.15	0.15	0.14
Hardness (as CaCO ₃)	130.2	1	30	28	25
Nitrate (as N)	9056	0.05	<0.05	<0.05	<0.05
Nitrite (as N)	9056	0.05	<0.05	<0.05	<0.05
Ortho-Phosphate (as P)	9056	0.05	<0.05	<0.05	<0.05
pH	9040B	NA	6.3	6.3	6.3
Specific Conductance	9050	NA	140	140	140
Sulfate (as SO ₄)	9056	0.8	6.4	6.6	5.8
Total Cyanide (as CN)	9010B/9014	0.001	0.003	<0.001	0.024
Total Organic Carbon	9060	0.5	4.83	3.80	3.54
TOC Range	9060	NA	4.65-5.09	3.76-3.84	3.51-3.57
Total Suspended Solids	160.2	2	85	3	2

TABLE A-1 (CONTINUED) - METALS^c

Analyte	EPA Method	Detection Limits	March 16, 1998	March 18, 1998	March 20, 1998
Aluminum (Al)	6020	0.005	0.074	0.028	0.022
Antimony (Sb)	6020	0.0050	<0.0050	<0.0050	<0.0050
Arsenic (As)	6020	0.005	<0.005	<0.005	<0.005
Barium (Ba)	6020	0.0050	0.067	0.060	0.062
Beryllium (Be)	6020	0.0010	<0.0010	<0.0010	<0.0010
Cadmium (Cd)	6020	0.0005	<0.0005	<0.0005	<0.0005
Calcium (Ca)	6010B	0.5	6.0	6.0	5.8
Chromium (Cr)	6020	0.0020	0.004	0.004	0.004
Cobalt (Co)	6020	0.0050	<0.0050	<0.0050	<0.0050
Copper (Cu)	6020	0.0050	0.0050	<0.0050	<0.0050
Iron (Fe)	6010B	0.1	56	35	35
Lead (Pb)	6020	0.0050	<0.0050	<0.0050	<0.0050
Manganese (Mn)	6020	0.0050	0.97	1.0	1.0
Magnesium (Mg)	6010B	0.1	3.4	3.3	3.2
Mercury (Hg)	7470A	0.0002	<0.0002	<0.0002	<0.0002
Nickel (Ni)	6020	0.0050	<0.0050	<0.0050	<0.0050
Potassium (K)	6010B	0.1	1.1	1.1	1.1
Selenium (Se)	6020	0.005	<0.005	<0.005	<0.005

TABLE A-1. (CONTINUED) - METALS CONT^c

Analyte	EPA Method	Detection Limits	March 16, 1998	March 18, 1998	March 20, 1998
Sodium (Na)	6010B	0.5	11	11	11
Thallium (Tl)	6020	0.0020	<0.0020	<0.0020	<0.0020
Vanadium (V)	6020	0.0050	<0.0050	<0.0050	<0.0050
Zinc (Zn)	6010B	0.02	<0.02	<0.02	<0.02

TABLE A-1. (CONTINUED) - PRIORITY POLLUTANT VOLATILE ORGANICS^d

Analyte	EPA Method	Detection Limits	March 16, 1998	March 18, 1998	March 20, 1998
Acrolein	8260B	100	<100	<100	<100
Acrylonitrile	8260B	100	<100	<100	<100
Benzene	8260B	5	<5	<5	<5
Bromodichloromethane	8260B	5	<5	<5	<5
Bromoform	8260B	5	<5	<5	<5
Bromomethane	8260B	10	<10	<10	<10
Carbon Tetrachloride	8260B	5	<5	<5	<5
Chlorobenzene	8260B	5	<5	<5	<5
Chloroethane	8260B	10	<10	<10	<10
2-Chloroethylvinyl Ether	8260B	10	<10	<10	<10
Chloroform	8260B	5	<5	<5	<5
Chloromethane	8260B	10	<10	<10	<10
Dibromochloromethane	8260B	5	<5	<5	<5
1,1-Dichloroethane	8260B	5	<5	<5	<5
1,1-Dichloroethane	8260B	5	<5	<5	<5
1,2-Dichloroethane	8260B	5	<5	<5	<5
trans-1,2-Dichloroethane	8260B	5	<5	<5	<5
1,2-Dichloropropane	8260B	5	<5	<5	<5

TABLE A-1. (CONTINUED) - PRIORITY POLLUTANT VOLATILE ORGANICS CONT'D

Analyte	EPA Method	Detection Limits	March 16, 1998	March 18, 1998	March 20, 1998
cis-1,3-Dichloropropene	8260B	5	<5	<5	<5
trans-1,3-Dichloropropene	8260B	5	<5	<5	<5
Ethylbenzene	8260B	5	<5	<5	<5
Methylene Chloride	8260B	5	<5	<5	<5
Tetrachloroethene	8260B	5	<5	<5	<5
1,1,2,2-Tetrachloroethane	8260B	5	<5	<5	<5
Toluene	8260B	5	<5	<5	<5
Trichloroethene	8260B	5	<5	<5	<5
1,1,1-Trichloroethane	8260B	5	<5	<5	<5
1,1,2-Trichloroethane	8260B	5	<5	<5	<5
Trichlorofluoromethane	8260B	5	<5	<5	<5
Vinyl Chloride	8260B	10	29	29	32

TABLE A-1. (CONTINUED) - PRIORITY POLLUTANT BASE NEUTRALS^d

Analyte	EPA Method	Detection Limits	March 16, 1998	March 18, 1998	March 20, 1998
Acenaphthene	8270C	10	<10	<10	<10
Acenaphthylene	8270C	10	<10	<10	<10
Anthracene	8270C	10	<10	<10	<10
Benzidine	8270C	50	<50	<50	<50
Benzo(a) Anthracene	8270C	10	<10	<10	<10
Benzo(b) Fluoranthene	8270C	10	<10	<10	<10
Benzo(k) Fluoranthene	8270C	10	<10	<10	<10
Benzo(g,h,i) Perylene	8270C	10	<10	<10	<10
Benzo(a) Pyrene	8270C	10	<10	<10	<10
Bis(2-Chloroethoxy) Methane	8270C	10	<10	<10	<10
Bis(2-Chloroethyl) Ether	8270C	10	<10	<10	<10
Bis(2-Chloroisopropyl) Ether	8270C	10	<10	<10	<10
Bis(2-Ethylhexyl) Phthalate	8270C	10	<10	<10	<10
4-Bromophenyl Phenyl Ether	8270C	10	<10	<10	<10
Butyl Benzyl Phthalate	8270C	10	<10	<10	<10
2-Chloronaphthalene	8270C	10	<10	<10	<10
4-Chlorophenyl Phenyl Ether	8270C	10	<10	<10	<10
Chrysene	8270C	10	<10	<10	<10
Dibenzo (a,h) Anthracene	8270C	10	<10	<10	<10
1,2-Dichlorobenzene	8270C	10	<10	<10	<10

TABLE A-1. (CONTINUED) - PRIORITY POLLUTANT BASE NEUTRALS CONT'D

Analyte	EPA Method	Detection Limits	March 16, 1998	March 18, 1998	March 20, 1998
1,3-Dichlorobenzene	8270C	10	<10	<10	<10
1,4-Dichlorobenzene	8270C	10	<10	<10	<10
3,3'-Dichlorobenzidine	8270C	20	<20	<20	<20
Dimethyl Phthalate	8270C	10	<10	<10	<10
Diethyl Phthalate	8270C	10	<10	<10	<10
Di-n-Butyl Phthalate	8270C	10	<10	<10	<10
Di-n-Octyl Phthalate	8270C	10	<10	<10	<10
2,4-Dinitrotoluene	8270C	10	<10	<10	<10
2,6-Dinitrotoluene	8270C	10	<10	<10	<10
Fluoranthene	8270C	10	<10	<10	<10
Fluorene	8270C	10	<10	<10	<10
Hexachlorobenzene	8270C	10	<10	<10	<10
Hexachloroethane	8270C	10	<10	<10	<10
Hexachlorobutadiene	8270C	10	<10	<10	<10
Hexachlorocyclopentadiene	8270C	10	<10	<10	<10
Indeno(1,2,3-cd) Pyrene	8270C	10	<10	<10	<10
Isophorone	8270C	10	<10	<10	<10

TABLE A-1. (CONTINUED) - PRIORITY POLLUTANT BASE NEUTRALS CON'T^d

Analyte	EPA Method	Detection Limits	March 16, 1998	March 18, 1998	March 20, 1998
Naphthalene	8270C	10	<10	<10	<10
Nitrobenzene	8270C	10	<10	<10	<10
N-Nitrosodimethylamine	8270C	10	<10	<10	<10
N-Nitrosodiphenylamine	8270C	10	<10	<10	<10
N-Nitrosodi-n-propylamine	8270C	10	<10	<10	<10
Phenanthrene	8270C	10	<10	<10	<10
Pyrene	8270C	10	<10	<10	<10
1,2,4-Trichlorobenzene	8270C	10	<10	<10	<10

TABLE A-1. (CONTINUED) - PRIORITY POLLUTANT ACID EXTRACTABLES^d

Analyte	EPA Method	Detection Limits	March 16, 1998	March 18, 1998	March 20, 1998
4-Chloro-3-Methyl Phenol	8270C	20	<20	<20	<20
2-Chlorophenol	8270C	10	<10	<10	<10
2,4-Dichlorophenol	8270C	10	<10	<10	<10
2,4-Dimethylphenol	8270C	10	<10	<10	<10
4,6-Dinitro-2-Methyl Phenol	8270C	50	<50	<50	<50
2,4-Dinitrophenol	8270C	50	<50	<50	<50
2-Nitrophenol	8270C	10	<10	<10	<10
4-Nitrophenol	8270C	50	<50	<50	<50
Pentachlorophenol	8270C	50	<50	<50	<50
Phenol	8270C	10	<10	<10	<10
2,4,6-Trichlorophenol	8270C	10	<10	<10	<10

TABLE A-1. (CONTINUED) - PRIORITY POLLUTANT ORGANOPHOSPHORUS PESTICIDES^d

Analyte	EPA Method	Detection Limits	March 16, 1998	March 18, 1998	March 20, 1998
Azinphos-methyl (Guthion)	8141A	1.0	ND	ND	ND
Baytex (Fenthion)	8141A	1.0	ND	ND	ND
Bolstar	8141A	1.0	ND	ND	ND
Coumaphos	8141A	1.0	ND	ND	ND
Demeton O&S	8141A	1.0	ND		
	8141A	10		ND	ND
Diazinon	8141A	1.0	ND		
	8141A	10		ND	ND
Dichlorvos	8141A	20	ND	ND	ND
Disyston (Disulfoton)	8141A	1.0			ND
	8141A	10	ND	ND	
Dursban (Chlorpyrifos)	8141A	1.0	ND	ND	ND
Ethoprop	8141A	1.0			ND
	8141A	10	ND	ND	
Merphos	8141A	1.0	ND	ND	ND
Methyl parathion	8141A	0.50	ND	ND	ND
Phorate (Thimet)	8141A	10	ND	ND	ND
Ronnel	8141A	1.0	ND	ND	ND
Tetrachlovinophos	8141A	1.0	ND	ND	ND
Tokuthion (Propiophos)	8141A	1.0	ND	ND	ND
Trichloronate	8141A	1.0	ND	ND	ND

TABLE A-1. (CONTINUED) - CHLORINATED PESTICIDES AND HERBICIDES^d

Analyte	EPA Method	Detection Limits	March 16, 1998	March 18, 1998	March 20, 1998
<u>Pesticides:</u>					
Aldrin	8081A/8082	0.05	<0.05	<0.05	<0.05
α -BHC	8081A/8082	0.05	<0.05	<0.05	<0.05
β -BHC	8081A/8082	0.05	<0.05	<0.05	<0.05
γ -BHC (Lindane)	8081A/8082	0.05	<0.05	<0.05	<0.05
δ -BHC	8081A/8082	0.05	<0.05	<0.05	<0.05
4,4'-DDD	8081A/8082	0.3	<0.3	<0.3	<0.3
4,4'-DDE	8081A/8082	0.1	<0.1	<0.1	<0.1
4,4'-DDT	8081A/8082	0.3	<0.3	<0.3	<0.3
Dieldrin	8081A/8082	0.1	<0.1	<0.1	<0.1
Endosulfan I	8081A/8082	0.1	<0.1	<0.1	<0.1
Endosulfan II	8081A/8082	0.3	<0.3	<0.3	<0.3
Endosulfan Sulfate	8081A/8082	0.3	<0.3	<0.3	<0.3
Endrin	8081A/8082	0.1	<0.1	<0.1	<0.1
Endrin Aldehyde	8081A/8082	0.3	<0.3	<0.3	<0.3
Heptachlor	8081A/8082	0.05	<0.05	<0.05	<0.05
Heptachlor Epoxide	8081A/8082	0.05	<0.05	<0.05	<0.05
Technical Chlordane	8081A/8082	1	<1	<1	<1
Total PCBs	8081A/8082	1	<1	<1	<1

TABLE A-1. (CONTINUED) - CHLORINATED PESTICIDES AND HERBICIDES CONT^d

Analyte	EPA Method	Detection Limits	March 16, 1998	March 18, 1998	March 20, 1998
Toxaphene	8081A/8082	3	<3	<3	<3
<u>Herbicides:</u>					
2,4-D	8151A	0.5	<0.5	<0.5	<0.5
2,4,5-TP (Silvex)	8151A	0.2	<0.2	<0.2	<0.2

TABLE A-1. (CONTINUED) - EXPLOSIVES^d

Analyte	EPA Method	Detection Limits	March 16, 1998	March 18, 1998	March 20, 1998
2-Amino-4,6-dinitrotoluene (2-AM-4,6 DNT)	8330	50	BDL	BDL	BDL
4-Amino-4,6-dinitrotoluene (4-AM-4,6-DNT)	8330	50	BDL	BDL	BDL
1,3-Dinitrobenzene (1,3-DNB)	8330	50	BDL	BDL	BDL
2,4-Dinitrotoluene (2,4-DNT)	8330	50	BDL	BDL	BDL
2,6-Dinitro-toluene (2,6-DNT)	8330	50	BDL	BDL	BDL
Hexahydro-1,3,5-trinitro-1,3,4-triazine (RDX)	8330	50	BDL	BDL	BDL
N,2,4,6-tetranitro-N-methylaniline (tetryl)	8330	50	BDL	BDL	BDL
Nitrobenzene (NB)	8330	50	BDL	BDL	BDL
2-Nitrotoluene (2-NT)	8330	50	BDL	BDL	BDL
3-Nitrotoluene (3-NT)	8330	50	BDL	BDL	BDL
4-Nitrotoluene (4-NT)	8330	50	BDL	BDL	BDL
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)	8330	50	BDL	BDL	BDL
1,3,5-Trinitrobenzene (TNB)	8330	50	BDL	BDL	BDL
Trinitrotoluene (TNT)	8330	50	BDL	BDL	BDL

TABLE A-1. (CONTINUED) - CHEMICAL AGENT^d

Analyte	EPA Method	Detection Limits	March 16, 1998	March 18, 1998	March 20, 1998
Diisopropyl methylphosphonate (DIMP)	^e	250	6,050	5090	4,720

^a All results expressed as mg/L except for specific conductance and pH which are expressed as $\mu\text{mhos/cm}$ and standard units, respectively.

^b Standard Methods 4500-NH₃E (APHA et al., 1995).

^c All metals expressed as mg/L total metal.

^d Results expressed as $\mu\text{g/L}$.

^e GPL Laboratories, LLLP (1998).

APPENDIX 2

GREEN ALGAL 96-H GROWTH TEST CONDUCTED ON GROUNDWATER TAKEN FROM CANAL CREEK WELL NO. CCJ153A

Test Method:	EPA/600/4-91/002 (Lewis et al., 1994)
Type of Test:	Static non-renewal
Date:	March 17-21, 1998
Investigator:	S. D. Turley
Laboratory:	UMD/WREC
Groundwater:	
Source:	Canal Creek Well No. CCJ153A
Chemical Characteristics:	See Appendix 1
Test Medium:	Stock culture medium
Test Organism:	
Scientific Name:	<i>Selenastrum capricornutum</i>
Age at Start of Test:	Log growth
Source:	UMD/WREC culture
Experimental Chambers:	
Material:	250 mL glass culture flasks with cheesecloth/cotton stoppers
Test Solution Volume:	100 mL
Initial Cell Density:	$\sim 1 \times 10^4$ cells/mL
No. Replicates per Treatment:	3
Lighting:	Fluorescent; cool white; continuous; ~ 300 foot candles
Shaking Rate:	100 cpm continuously
Endpoint:	Reduction in growth relative to control
Test Temperature:	25 ± 0.1 °C
Water Quality:	Table A2-1

Results:

The groundwater did not affect the growth (cell density) of algae after 96 hours of exposure (Tables A2-2 and A2-3).

Table A2-1. SUMMARY OF THE CANAL CREEK GROUNDWATER (WELL NO. CCJ153A) TOXICITY TEST WATER QUALITY FOR THE GREEN ALGAL 96-H GROWTH TEST - pH (STANDARD UNITS)

	Test Concentrations (Percent Groundwater by Volume)					
	0	10	18	32	56	100
<u>Day 0</u>						
0 H	7.70	7.72	7.76	7.72	7.70	7.67
<u>Day 1</u>						
24 H	7.77	7.79	7.73	7.70	7.69	7.72
<u>Day 2</u>						
24 H	7.69	7.71	7.62	7.64	7.60	7.65
<u>Day 3</u>						
24 H	7.65	7.59	7.60	7.66	7.64	7.68
<u>Day 4</u>						
24 H	7.60	7.56	7.59	7.61	7.58	7.55
Min	7.60	7.56	7.59	7.61	7.58	7.55
Max	7.77	7.79	7.76	7.72	7.70	7.72

Table A2-2 GREEN ALGAL CANAL CREEK GROUNDWATER (WELL NO. CCJ153A) TOXICITY TEST DATA - MEAN CELL DENSITY (CELLS/ML) AFTER 96 HOURS OF EXPOSURE

Concentration (% by Volume)	Rep	Mean Cell Density	
		0 H	96 H
Growth Medium	1	10000	1225000
	2	10000	1308000
	3	10000	1292000
10	1	10000	1321000
	2	10000	1200000
	3	10000	1290000
18	1	10000	1290000
	2	10000	1230000
	3	10000	1219000
32	1	10000	1340000
	2	10000	1286000
	3	10000	1328000
56	1	10000	1220000
	2	10000	1211000
	3	10000	1286000
100	1	10000	1160000
	2	10000	1205000
	3	10000	1225000

Table A2-3. GREEN ALGAL CANAL CREEK GROUNDWATER (WELL NO. CCJ153A) TOXICITY TEST STATISTICAL ANALYSIS - MEAN CELL DENSITY (CELLS/ML)

Data Transformation:

No transformation

Shapiro-Wilk's Test for Normality:

Calculated test statistic:	0.95
Alpha value:	0.01
Critical value:	0.86
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test Homogeneity of Variances:

Calculated test statistic:	1.32
Alpha value:	0.01
Critical value:	15.09
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	2.73
Alpha value:	0.05
Critical value:	3.11
Conclusion:	Fail to reject the null hypothesis that all groups are equal

APPENDIX 3

CLADOCERAN ACUTE AND 7-DAY SURVIVAL AND REPRODUCTION TEST CONDUCTED ON GROUNDWATER TAKEN FROM CANAL CREEK WELL NO. CCJ153A

Test Method:	EPA/600/4-91/002 (Lewis et al., 1994)
Type of Test:	Static renewal (every 24 h)
Date:	March 16-23, 1998
Investigator:	S. D. Turley
Laboratory:	UMD/WREC
Groundwater:	
Source:	Canal Creek Well No. CCJ153A
Chemical Characteristics:	See Appendix 1
Dilution Water:	
Source:	20% Perrier:80% RO Water
Chemical Characteristics:	See Appendix A3-1
Test Organism:	
Scientific Name:	<i>Ceriodaphnia dubia</i>
Age at Start of Test:	<4 h
Source:	UMD/WREC culture
Experimental Chambers:	
Material:	50 mL glass beaker
Test Solution Volume:	25 mL
No. Organisms/Replicate:	1
No. Organisms/Treatment:	10
Feeding:	0.1 mL algal suspension and 0.24 mL digested Cerophyl®/test chamber daily
Loading:	1 organism/beaker
Lighting:	Fluorescent; 60-85 foot candles
Aeration:	Diluent water only prior to each renewal
Endpoints:	Mortality of adults; number of neonates produced in 3 broods
Test Temperature:	25 ± 0.1 °C

Water Quality:

Table A3-1

Results:

Mortality:

Canal Creek groundwater from well no. CCJ153A had no effect on the acute (48 h) or chronic survival of the cladocerans after 7 d of exposure (Tables A3-2 and A3-3).

Neonate Production:

The groundwater had no affect ($\alpha = 0.05$) on neonate production after 7 d of exposure (Tables A3-3 and A3-4).

Table A3-1. SUMMARY OF THE CANAL CREEK GROUNDWATER (WELL NO. CCJ153A) TOXICITY TEST WATER QUALITY DATA FOR THE CLADOCERAN 7-DAY TEST - DISSOLVED OXYGEN (MG/L)

	Test Concentrations (Percent Groundwater by Volume)					
	0	10	18	32	56	100
<u>Day 0</u>						
0 H	8.3	8.3	8.3	8.2	7.7	7.1
<u>Day 1</u>						
0 H	8.3	8.3	8.3	8.2	7.9	7.3
24 H	7.6	7.5	7.6	7.5	7.5	7.5
<u>Day 2</u>						
0 H	8.2	8.2	8.2	8.1	8.0	7.0
24 H	8.1	7.9	7.7	7.7	7.6	7.6
<u>Day 3</u>						
0 H	8.3	8.3	8.3	8.2	8.1	7.5
24 H	8.0	8.1	8.2	8.0	7.5	7.3
<u>Day 4</u>						
0 H	8.3	8.3	8.3	8.3	8.1	7.3
24 H	8.0	8.1	8.0	7.4	7.5	7.4
<u>Day 5</u>						
0 H	8.3	8.3	8.2	8.2	8.0	7.4
24 H	8.0	8.0	8.0	7.4	7.4	7.5
<u>Day 6</u>						
0 H	8.3	8.3	8.3	8.3	8.0	8.0
24 H	8.0	8.0	8.1	7.6	7.5	7.6
<u>Day 7</u>						
24 H	7.9	7.9	8.0	7.5	7.4	7.5
Mean	8.1	8.1	8.1	7.9	7.7	7.4
Min	7.6	7.5	7.6	7.4	7.4	7.0
Max	8.3	8.3	8.3	8.3	8.1	8.0

Table A3-1. (CONTINUED) - pH (STANDARD UNITS)

	Test Concentrations (Percent Groundwater by Volume)					
	0	10	18	32	56	100
<u>Day 0</u>						
0 H	7.19	7.12	7.03	6.88	6.78	6.30
<u>Day 1</u>						
0 H	7.32	7.18	7.27	7.36	7.41	7.00
24 H	6.92	7.04	7.08	7.13	7.22	7.41
<u>Day 2</u>						
0 H	7.03	7.04	7.05	7.01	6.97	6.55
24 H	6.90	6.99	7.05	7.21	7.27	7.40
<u>Day 3</u>						
0 H	7.00	6.99	7.02	7.02	6.98	6.70
24 H	6.98	7.10	7.11	7.12	7.11	7.12
<u>Day 4</u>						
0 H	7.08	7.11	7.12	7.08	6.97	6.77
24 H	7.04	7.24	7.29	7.31	7.33	7.45
<u>Day 5</u>						
0 H	7.11	7.15	7.17	7.10	6.93	6.69
24 H	7.00	7.13	7.17	7.23	7.29	7.37
<u>Day 6</u>						
0 H	6.99	7.05	7.07	7.04	6.93	6.89
24 H	6.95	7.11	7.15	7.19	7.20	7.29
<u>Day 7</u>						
24 H	7.02	7.12	7.12	7.15	7.22	7.30
Min	6.90	6.99	7.02	6.88	6.78	6.30
Max	7.32	7.24	7.29	7.36	7.41	7.45

Table A3-1. (CONTINUED) - CONDUCTIVITY(μ MHOS/CM)

	Test concentrations (Percent Groundwater by Volume)	
	0	100
<u>Day 0</u>		
0 H	165	1000
<u>Day 1</u>		
0 H	170	1050
<u>Day 2</u>		
0 H	170	1000
<u>Day 3</u>		
0 H	160	1000
<u>Day 4</u>		
0 H	170	1100
<u>Day 5</u>		
0 H	170	1050
<u>Day 6</u>		
0 H	160	1000
<u>Day 7</u>		
24 H	160	1000
Mean	166	1025
Min	160	1000
Max	170	1100

Table A3-1. (CONTINUED) - ALKALINITY (MG/L AS CaCO₃)

	Test Concentrations (Percent Groundwater by Volume)	
	0	100
<u>Day 0</u>		
0 H	30	60
<u>Day 1</u>		
0 H	30	65
<u>Day 2</u>		
0 H	30	65
<u>Day 3</u>		
0 H	35	65
<u>Day 4</u>		
0 H	30	60
<u>Day 5</u>		
0 H	35	60
<u>Day 6</u>		
0 H	30	65
<u>Day 7</u>		
24	30	60
Mean	31	63
Min	30	60
Max	35	65

Table A3-1. (CONTINUED) - HARDNESS (MG/L AS CaCO₃)

	Test Concentrations (Percent Groundwater by Volume)	
	0	100
<u>Day 0</u>		
0 H	80	40
<u>Day 1</u>		
0 H	80	40
<u>Day 2</u>		
0 H	84	40
<u>Day 3</u>		
0 H	80	40
<u>Day 4</u>		
0 H	80	35
<u>Day 5</u>		
0 H	80	40
<u>Day 6</u>		
0 H	84	35
<u>Day 6</u>		
24	80	40
Mean	81	39
Min	80	35
Max	84	40

Table A3-2. CLADOCERAN CANAL CREEK GROUNDWATER (WELL NO. CCJ153A) TOXICITY TEST DATA - SURVIVAL AFTER 48 HOURS OF EXPOSURE

Concentration (% by Volume)	Number Tested	No. Alive at 48 Hours	Percent Alive
Control	10	10	100
10	10	10	100
18	10	10	100
32	10	10	100
56	10	10	100
100	10	10	100

Table A3-3. CLADOCERAN CANAL CREEK GROUNDWATER (WELL NO. CCJ153A) TOXICITY TEST DATA - SURVIVAL OF ADULTS, TOTAL NUMBER OF YOUNG, AND NUMBER OF YOUNG PRODUCED PER BROOD AFTER 7 DAYS OF EXPOSURE

Concentration (% by Volume)	Rep	Brood No. 1	Brood No. 2	Brood No. 3	Total Young
Control	1	3	9	14	26
	2	5	8	14	27
	3	4	9	15	28
	4	4	8	13	25
	5	5	8	13	26
	6	3	10	14	27
	7	5	9	15	29
	8	4	9	12	25
	9	4	8	15	27
	10	5	9	16	30
10	1	5	9	14	28
	2	5	10	15	30
	3	4	9	13	26
	4	4	8	16	28
	5	3	9	12	24
	6	4	7	15	26
	7	4	10	14	28
	8	5	8	13	26
	9	4	8	14	26
	10	3	9	14	26

TABLE A3-3. (CONTINUED)

Concentration (% by Volume)	Rep	Brood No. 1	Brood No. 2	Brood No. 3	Total Young
18	1	3	9	15	27
	2	3	9	12	24
	3	4	7	15	26
	4	5	8	14	27
	5	5	10	14	29
	6	5	8	13	26
	7	4	8	13	25
	8	4	7	14	25
	9	5	9	15	29
	10	4	9	12	25
32	1	4	7	15	26
	2	3	9	14	26
	3	4	9	13	26
	4	4	10	15	29
	5	4	8	12	24
	6	5	9	13	27
	7	4	7	16	27
	8	4	9	13	26
	9	5	8	14	27
	10	4	8	15	27

TABLE A3-3. (CONTINUED)

Concentration (% by Volume)	Rep	Brood No. 1	Brood No. 2	Brood No. 3	Total Young
56	1	4	9	13	26
	2	4	9	15	28
	3	4	7	14	25
	4	5	7	14	26
	5	3	8	13	24
	6	4	8	13	25
	7	5	8	15	28
	8	4	8	12	24
	9	4	9	13	26
	10	5	8	14	27
100	1	4	7	15	26
	2	5	9	12	26
	3	4	7	11	22
	4	4	8	15	27
	5	5	10	14	29
	6	3	7	13	23
	7	5	8	13	26
	8	4	8	14	26
	9	4	8	12	24
	10	4	8	13	25

Table A3-4. CLADOCERAN CANAL CREEK GROUNDWATER (WELL NO. CCJ153A) TOXICITY TEST STATISTICAL ANALYSIS - NEONATE PRODUCTION AFTER 7 DAYS OF EXPOSURE

Data Transformation:

None

Shapiro-Wilk's Test for Normality:

This test could not be performed because total number of replicates was greater than 50.

Chi-Square Test for Normality:

Calculated test statistic:	4.48
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test Homogeneity of Variances:

Calculated test statistic:	2.07
Alpha value:	0.01
Critical value:	15.09
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	1.30
Alpha value:	0.05
Critical value:	2.45
Conclusion:	Fail to reject the null hypothesis that all groups are equal

APPENDIX 4

FATHEAD MINNOW ACUTE AND CHRONIC 7-DAY SURVIVAL AND GROWTH TEST CONDUCTED ON GROUNDWATER TAKEN FROM CANAL CREEK WELL NO. CCJ153A

Test Method:	EPA/600/4-91/002 (Lewis et al., 1994)
Type of Test:	Static renewal (every 24 h)
Date:	March 16-23, 1998
Investigator:	S. D. Turley
Laboratory:	UMD/WREC
Groundwater:	
Source:	Canal Creek Well No. CCJ153A
Chemical Characteristics:	See Appendix 1
Dilution Water:	
Source:	20% Perrier:80% RO Water
Chemical Characteristics:	Table A4-1
Test Organism:	
Scientific Name:	<i>Pimephales promelas</i>
Age at Start of Test:	<24 h
Source:	UMD/WREC culture
Experimental Chambers:	
Material:	600 mL glass beaker
Test Solution Volume:	400 mL
No. Organisms/Replicate:	10
No. Organisms/Treatment:	40
Loading:	<0.5 g/L
Feeding:	Concentrated stock of 0.1 mL <i>Artemia</i> nauplii three times daily
Lighting:	Fluorescent; 60-85 foot candles
Aeration:	Diluent water only prior to each renewal
Endpoints:	Mortality; growth
Test Temperature:	25 ± 0.1 °C
Water Quality:	Table A4-1

Results:

Survival:

The groundwater had no acute or short-term chronic effect on the survival of fathead minnow larvae (Tables A4-2, A4-3, and A4-4). Survival was 90% or greater in all test treatments after 7 d of exposure.

Growth:

The groundwater had no effect on fathead minnow larval growth after 7 d of exposure (Tables A4-3 and A4-5).

Table A4-1. SUMMARY OF THE CANAL CREEK GROUNDWATER (WELL NO. CCJ153A) BIOASSAY WATER QUALITY DATA FOR THE FATHEAD MINNOW 7-DAY TEST - DISSOLVED OXYGEN (MG/L)

	Test Concentrations (Percent Groundwater by Volume)					
	0	10	18	32	56	100
<u>Day 0</u>						
0 H	8.3	8.3	8.3	8.2	7.7	7.1
<u>Day 1</u>						
0 H	8.3	8.3	8.3	8.2	7.9	7.3
24 H	8.1	8.0	7.8	7.9	7.9	7.7
<u>Day 2</u>						
0 H	8.2	8.2	8.2	8.1	8.0	7.0
24 H	7.8	7.5	7.4	7.3	7.4	7.4
<u>Day 3</u>						
0 H	8.3	8.3	8.3	8.2	8.1	7.5
24 H	7.2	7.2	7.4	7.2	7.2	7.4
<u>Day 4</u>						
0 H	8.3	8.3	8.3	8.3	8.1	7.3
24 H	7.5	7.5	7.4	7.4	7.5	7.4
<u>Day 5</u>						
0 H	8.3	8.3	8.2	8.2	8.0	7.4
24 H	7.4	7.5	7.5	7.3	7.3	7.3
<u>Day 6</u>						
0 H	8.3	8.3	8.3	8.3	8.0	8.0
24 H	7.3	7.4	7.4	7.2	7.2	7.2
<u>Day 7</u>						
24 H	7.3	7.3	7.3	7.2	7.2	7.1
Mean	7.9	7.9	7.9	7.8	7.7	7.4
Min	7.2	7.2	7.3	7.2	7.2	7.0
Max	8.3	8.3	8.3	8.3	8.1	8.0

Table A4-1. (CONTINUED) - pH (STANDARD UNITS)

	Test Concentrations (Percent Groundwater by Volume)					
	0	10	18	32	56	100
<u>Day 0</u>						
0 H	7.19	7.12	7.03	6.88	6.78	6.30
<u>Day 1</u>						
0 H	7.32	7.18	7.27	7.36	7.41	7.00
24 H	7.15	7.13	7.13	7.18	7.20	7.22
<u>Day 2</u>						
0 H	7.03	7.04	7.05	7.01	6.97	6.55
24 H	7.19	6.85	6.87	6.99	7.14	7.21
<u>Day 3</u>						
0 H	7.00	6.99	7.02	7.02	6.98	6.70
24 H	6.80	6.83	6.83	6.88	7.03	7.09
<u>Day 4</u>						
0 H	7.08	7.11	7.12	7.08	6.97	6.77
24 H	7.13	7.09	7.06	7.04	7.11	7.19
<u>Day 5</u>						
0 H	7.11	7.15	7.17	7.10	6.93	6.69
24 H	7.04	7.02	6.99	7.08	7.14	7.24
<u>Day 6</u>						
0 H	6.99	7.05	7.07	7.04	6.93	6.89
24 H	7.09	7.03	7.01	7.11	7.19	7.21
<u>Day 7</u>						
24 H	7.02	7.05	7.00	7.12	7.16	7.19
Min	6.80	6.83	6.83	6.88	6.78	6.30
Max	7.32	7.18	7.27	7.36	7.41	7.24

Table A4-1. (CONTINUED) - CONDUCTIVITY(μ MHOS/CM)

	Test concentrations (Percent Groundwater by Volume)	
	0	100
<u>Day 0</u>		
0 H	165	1000
<u>Day 1</u>		
0 H	170	1050
<u>Day 2</u>		
0 H	170	1000
<u>Day 3</u>		
0 H	160	1000
<u>Day 4</u>		
0 H	170	1100
<u>Day 5</u>		
0 H	170	1050
<u>Day 6</u>		
0 H	160	1000
<u>Day 7</u>		
24 H	160	1000
Mean	166	1025
Min	160	1000
Max	170	1100

Table A4-1. (CONTINUED) - ALKALINITY (MG/L AS CaCO₃)

	Test Concentrations (Percent Groundwater by Volume)	
	0	100
<u>Day 0</u>		
0 H	30	60
<u>Day 1</u>		
0 H	30	65
<u>Day 2</u>		
0 H	30	65
<u>Day 3</u>		
0 H	35	65
<u>Day 4</u>		
0 H	30	60
<u>Day 5</u>		
0 H	35	60
<u>Day 6</u>		
0 H	30	65
<u>Day 7</u>		
24 H	30	60
Mean	31	62
Min	30	60
Max	35	65

Table A4-1. (CONTINUED) - HARDNESS (MG/L AS CaCO₃)

	Test Concentrations (Percent Groundwater by Volume)	
	0	100
<u>Day 0</u>		
0 H	80	40
<u>Day 1</u>		
0 H	80	40
<u>Day 2</u>		
0 H	84	40
<u>Day 3</u>		
0 H	80	40
<u>Day 4</u>		
0 H	80	35
<u>Day 5</u>		
0 H	80	40
<u>Day 6</u>		
0 H	84	35
<u>Day 7</u>		
24 H	80	40
Mean	81	39
Min	80	35
Max	84	40

Table A4-2. FATHEAD MINNOW CANAL CREEK GROUNDWATER (WELL NO. CCJ153A) TOXICITY TEST DATA - SURVIVAL AFTER 96 H OF EXPOSURE

Concentration (% by Volume)	Rep	Number Tested	No. Alive at end of test	Percent Alive
Control	A	10	10	100
	B	10	10	100
	C	10	10	100
	D	10	10	100
10	A	10	10	100
	B	10	10	100
	C	10	10	100
	D	10	10	100
18	A	10	10	100
	B	10	10	100
	C	10	10	100
	D	10	10	100
32	A	10	10	100
	B	10	10	100
	C	10	10	100
	D	10	10	100
56	A	10	10	100
	B	10	10	100
	C	10	10	100
	D	10	10	100
100	A	10	10	100
	B	10	10	100
	C	10	10	100
	D	10	10	100

Table A4-3. FATHEAD MINNOW CANAL CREEK GROUNDWATER (WELL NO. CCJ153AA) TOXICITY TEST DATA - LARVAL SURVIVAL AND GROWTH AFTER 7 DAYS OF EXPOSURE

Concentration (% by Volume)	Rep	Number Larvae Alive	Percent Survival	Dry Weight ^a (mg)	Mean Dry Weight (mg)
Control	1	9	90	0.587	0.632
	2	10	100	0.629	
	3	9	90	0.607	
	4	10	100	0.706	
10	1	9	90	0.566	0.639
	2	10	100	0.629	
	3	10	100	0.722	
	4	10	100	0.639	
18	1	10	100	0.668	0.634
	2	9	90	0.639	
	3	10	100	0.653	
	4	9	90	0.576	
32	1	10	100	0.568	0.615
	2	10	100	0.622	
	3	10	100	0.639	
	4	10	100	0.631	
56	1	10	100	0.591	0.578
	2	10	100	0.565	
	3	9	90	0.495	
	4	10	100	0.660	
100	1	10	100	0.595	0.520
	2	10	100	0.519	
	3	9	90	0.423	
	4	10	100	0.543	

^a Dry weight = Total dry weight of larvae/number of original larvae (10)

Table A4-4. FATHEAD MINNOW CANAL CREEK GROUNDWATER (WELL NO. CCJ153A) TOXICITY TEST STATISTICAL ANALYSIS -SURVIVAL OF LARVAE AFTER 7 DAYS OF EXPOSURE

Data Transformation:

Arc-sine square root

Shapiro-Wilk's Test for Normality:

Calculated test statistic:	0.84
Alpha value:	0.01
Critical value:	0.88
Conclusion:	Reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Conclusion:	Data failed to meet the homogeneity of variance assumption
-------------	--

Steel's Many-One Ranked Test:

Ranked Sum statistics:	18.0 - 22.0
Alpha value:	0.05
Critical value:	10.0
Conclusion:	Fail to reject the null hypothesis that the groups are equal

Table A4-5. FATHEAD MINNOW CANAL CREEK GROUNDWATER (WELL NO. CCJ153A) TOXICITY TEST STATISTICAL ANALYSIS - GROWTH OF LARVAE AFTER 7 DAYS OF EXPOSURE

Data Transformation:

No transformation

Shapiro-Wilk's Test for Normality:

Calculated test statistic:	0.96
Alpha value:	0.01
Critical value:	0.88
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	2.40
Alpha value:	0.01
Critical value:	15.09
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	2.68
Alpha value:	0.05
Critical value:	2.77
Conclusion:	Fail to reject the null hypothesis that all groups are equal

APPENDIX 5

FROG EMBRYO TERATOGENESIS ASSAY - *XENOPUS* (FETAX) CONDUCTED ON GROUNDWATER TAKEN FROM CANAL CREEK WELL NO. CCJ153A

Test Method:	ASTM Designation E 1439-91 ASTM (1998)
Type of Test:	Static renewal (every 24 h)
Date:	March 17-21, 1998
Investigator:	S. D. Turley
Laboratory:	UMD/WREC
Groundwater:	
Source:	Canal Creek Well No. CCJ153A
Chemical Characteristics:	See Appendix 1
Test Medium:	FETAX solution
Test Organism:	
Scientific Name:	<i>Xenopus laevis</i>
Age at Start of Test:	Stage 8 blastula to stage 11 gastrula
Source:	UMD/WREC culture
Experimental Chambers:	
Material:	Glass petri dishes
Test Solution Volume:	10 mL
No. Organisms/Replicate:	25
No. Organisms/Treatment:	Control: 50 Groundwater: 50
Loading:	n/a
Lighting:	Fluorescent; 60-85 foot candles
Aeration:	Prior to renewals
Endpoints:	Mortality; malformation
Test Temperature:	25.1 ± 0.1 °C
Water Quality:	Table A5-1

Results:

Mortality:

The surficial groundwater from well CCJ153A was not acutely toxic to frog embryos after 96 h of exposure (Tables A5-2 and A5-3); thus, a LC50, NOAEL and LOAEL could not be calculated.

Malformations:

Statistically significant ($\alpha = 0.05$) embryo malformations occurred after 96 h of exposure (Tables A5-2, A5-4, and A5-5). The NOAEL and LOAEL are as follows:

NOAEL = 32 percent groundwater by volume

LOAEL = 56 percent groundwater by volume

The types of random malformed embryos are given in Table A5-6.

Table A5-1. SUMMARY OF THE FETAX CANAL CREEK GROUNDWATER (WELL NO. CCJ153A) BIOASSAY - pH (STANDARD UNITS)

	Test Concentrations (Percent Groundwater by Volume)					
	0	10	18	32	56	100
<u>Day 0</u>						
0H	7.60	7.55	7.46	7.32	7.19	7.00
<u>Day 1</u>						
0 H	7.65	7.60	7.47	7.39	7.16	6.55
<u>Day 2</u>						
0 H	7.67	7.57	7.41	7.35	7.12	6.70
<u>Day 3</u>						
0 H	7.63	7.51	7.40	7.37	7.10	6.77
<u>Day 4</u>						
24 H	7.43	7.26	7.25	7.29	7.14	6.99
Min	7.43	7.26	7.25	7.29	7.10	6.55
Max	7.67	7.60	7.47	7.39	7.19	7.00

Table A5-2. FETAX CANAL CREEK GROUNDWATER (WELL NO. CCJ153A)
TOXICITY TEST DATA - PERCENT EMBRYO SURVIVAL AND
MALFORMATIONS AFTER 96 HOURS OF EXPOSURE

Concentration (% by Volume)	Rep	Number Embryos Alive	Percent Survival	Number Embryos Malformed	Percent Malformed
Control	1	25	100	2	8.0
	2	23	92	1	4.3
10	1	25	100	2	8.0
	2	24	96	2	8.3
18	1	24	96	2	8.3
	2	23	92	3	13.0
32	1	23	92	2	8.7
	2	22	88	2	9.1
56	1	23	92	3	13.0
	2	24	96	3	12.5
100	1	22	88	3	13.6
	2	23	92	3	13.0

Table A5-3. FETAX CANAL CREEK GROUNDWATER (WELL NO. CCJ153A)
TOXICITY TEST STATISTICAL ANALYSIS - PERCENT EMBRYO
SURVIVAL AFTER 96 HOURS OF EXPOSURE

Data Transformation:

Arc-sine square root

Shapiro-Wilk's Test for Normality:

Statistic not run because only two replicates were used in the test as recommended in the test protocol. The data were assumed to be normally distributed. See Section 2.5 in the body of the report for further details.

Bartlett's Test for Homogeneity of Variances:

Statistic not run because only two replicates were used in the test as recommended in the test protocol. Homogeneity of variance was assumed to occur. See Section 2.5 in the body of the report for further details.

ANOVA:

Calculated test statistic:	1.60
Alpha value:	0.05
Critical value:	4.39
Conclusion:	Fail to reject the null hypothesis that all groups are equal

Table A5-4. FETAX CANAL CREEK GROUNDWATER (WELL NO. CCJ153A)
TOXICITY TEST STATISTICAL ANALYSIS - PERCENT
MALFORMATIONS AFTER 96 HOURS OF EXPOSURE

Data Transformation:

Arc-sine square root

Shapiro-Wilk's Test for Normality:

Statistic not run because only two replicates were used in the test as recommended in the test protocol. The data were assumed to be normally distributed. See Section 2.5 in the body of the report for further details.

Bartlett's Test for Homogeneity of Variances:

Statistic not run because only two replicates were used in the test as recommended in the test protocol. Homogeneity of variance was assumed to occur. See Section 2.5 in the body of the report for further details.

ANOVA:

Calculated test statistic:	4.50
Alpha value:	0.05
Critical value:	4.39
Conclusion:	Reject the null hypothesis that all groups are equal

Dunnett's Test:

Calculated test statistic:	See Table A5-5
Alpha value:	0.05
Critical value:	2.83
Conclusion:	Reject the null hypothesis that all groups are equal

Table A5-5. FETAX CANAL CREEK GROUNDWATER (WELL NO. CCJ153A)
TOXICITY TEST DATA - RESULTS OF DUNNETT'S TEST ON EMBRYO
MALFORMATIONS AFTER 96 H OF EXPOSURE

Concentration (% by Volume)	No. of Reps	Mean Survival (%) ^a	T Statistic	Significance
Control	2	93.9		
10	2	91.9	1.310	
18	2	89.4	2.600	
32	2	91.1	1.732	
56	2	87.3	3.686	*
100	2	86.7	3.943	*

^a Actual mean values are given in table. Transformed means were used in the statistical analysis.

* Significantly different at $\alpha = 0.05$ (Dunnett's critical value = 2.83).

Table A5-6. FETAX CANAL CREEK GROUNDWATER (WELL NO. CCJ153A)
TOXICITY TEST DATA - TYPE AND NUMBER OF MALFORMED
EMBRYOS AFTER 96 HOURS EXPOSURE

Malformation	Test Concentration (% Groundwater by Volume)												Tot No.
	0 Rep		10 Rep		18 Rep		32 Rep		56 Rep		100 Rep		
	1	2	1	2	1	2	1	2	1	2	1	2	
Severe										1	1		2
Gut coiling				1	1	2	2	1	2	1	2	1	13
Edema:													
Multiple													
Cardiac													
Abdominal	1	1	1		1	1			1	1		1	8
Facial													
Cephalic													
Blisters													
Tail													
Notochord	1		1	1				1				1	5
Fin													
Face													
Eye													
Brain													
Hemorrhage													
Cardiac													
Other													
Total No.	2	1	2	2	2	3	2	2	3	3	3	3	28

APPENDIX 6

GREEN ALGAL 96-H GROWTH TEST CONDUCTED WITH DIISOPROPYL METHYLPHOSPHONATE (DIMP)

Test Method:	EPA/600/4-91/002 (Lewis et al., 1994)
Type of Test:	Static non-renewal
Date:	July 8 - 12, 1998
Investigator:	S. D. Turley
Laboratory:	UMD/WREC
Toxicant:	Diisopropyl methylphosphonate
Source:	Reagent grade (Purity >99%)
Chemical Characteristics:	See Appendix 1
Test Medium:	Stock culture medium
Test Organism:	
Scientific Name:	<i>Selenastrum capricornutum</i>
Age at Start of Test:	Log growth
Source:	UMD/WREC culture
Experimental Chambers:	
Material:	250 mL glass culture flasks with cheesecloth/cotton stoppers
Test Solution Volume:	100 mL
Initial Cell Density:	$\sim 1 \times 10^4$ cells/mL
No. Replicates per Treatment:	3
Lighting:	Fluorescent; cool white; continuous; ~ 300 foot candles
Shaking Rate:	100 cpm continuously
Endpoint:	Reduction in growth relative to control
Water Quality:	Table A6-1

Results:

Cell Growth

Algal growth was significantly ($\alpha = 0.05$) reduced by 96-h exposure to DIMP at concentrations of 1,423, 2,845, and 5,690 mg/L (Tables A6-2, A6-3, and A6-4). A 96-h EC50, as determined by the Probit method, is as follows:

96-h EC50 = 3185 mg/L (95% confidence limits = 2,874.2 - 3,559.2)

NOAEL = 711 mg/L

LOAEL = 1,423 mg/L

TABLE A6-1. SUMMARY OF THE DIMP TOXICITY TEST WATER QUALITY DATA
FOR THE GREEN ALGAL 96-H GROWTH TEST - TEMPERATURE (°C)

	Test Concentrations (mg/L)					
	0	356	711	1423	2845	5690
<u>Day 0</u>						
0 H	25.0	25.0	25.0	25.0	25.0	25.0
<u>Day 4</u>						
96 H	25.0	25.0	25.0	25.0	25.0	25.0

TABLE A6-1. (CONTINUED) - pH (STANDARD UNITS)

	Test Concentrations (mg/L)					
	0	356	711	1423	2845	5690
<u>Day 0</u>						
0 H	7.65	7.58	7.55	7.47	7.42	7.38
<u>Day 4</u>						
96 H	7.74	7.76	7.79	7.86	7.99	8.11

TABLE A6-2. GREEN ALGAL TOXICITY TEST DATA - MEAN CELL DENSITY
(CELLS/ML) AFTER 96 HOURS OF EXPOSURE

Concentration (mg/L)	Rep	Mean Cell Density	
		0 H	96 H
Growth Medium	1	10100	1231000
	2	10100	1199000
	3	10100	1247000
356	1	10100	1295000
	2	10100	1259000
	3	10100	1232000
711	1	10100	1256000
	2	10100	1212000
	3	10100	1263000
1423	1	10100	1087000
	2	10100	1166000
	3	10100	1092000
2845	1	10100	611000
	2	10100	541000
	3	10100	576000
5690	1	10100	322000
	2	10100	299000
	3	10100	261000

TABLE A6-3. GREEN ALGAL DIMP TOXICITY TEST STATISTICAL ANALYSIS -
MEAN CELL DENSITY (CELLS/ML)

Data Transformation:

No transformation

Shapiro-Wilk's Test for Normality:

Calculated test statistic:	0.92
Alpha value:	0.01
Critical value:	0.86
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test Homogeneity of Variances:

Calculated test statistic:	0.73
Alpha value:	0.01
Critical value:	15.09
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	475.24
Alpha value:	0.05
Critical value:	3.11
Conclusion:	Reject the null hypothesis that all groups are equal

Dunnett's Test:

Calculated test statistic:	See Table A6-4
Alpha value:	0.05
Critical value:	2.50
Conclusion:	Reject the null hypothesis that all groups are equal

Table A6-4. GREEN ALGAL DIMP TOXICITY TEST STATISTICAL ANALYSIS -
RESULTS OF DUNNETT'S TEST ON MEAN CELL DENSITY
(CELLS/ML)

Concentration (mg/L)	No. of Reps	Mean Cell Density	T Statistic	Significance
Growth Medium	3	1225667		
356	3	1262000	-1.353	
711	3	1243667	-0.670	
1423	3	1115000	4.121	*
2845	3	576000	24.190	*
5690	3	294000	34.690	*

* Significantly different at alpha = 0.05 (Dunnett's critical value = 2.50).

APPENDIX 7

CLADOCERAN ACUTE AND 7-DAY SURVIVAL AND REPRODUCTION TEST CONDUCTED WITH DIISOPROPYL METHYLPHOSPHONATE (DIMP)

Test Method:	EPA/600/4-91/002 (Lewis et al., 1994)
Type of Test:	Static renewal (every 24 h)
Date:	July 8 - 15, 1998
Investigator:	S. D. Turley
Laboratory:	UMD/WREC
Toxicant:	Diisopropyl methylphosphonate
Source:	Reagent grade (Purity > 99%)
Chemical Characteristics:	See Appendix 1
Dilution Water:	
Source:	20% Perrier:80% RO Water
Chemical Characteristics:	Table A7-1
Test Organism:	
Scientific Name:	<i>Ceriodaphnia dubia</i>
Age at Start of Test:	<4 h
Source:	UMD/WREC culture
Experimental Chambers:	
Material:	50 mL glass beaker
Test Solution Volume:	25 mL
No. Organisms/Replicate:	1
No. Organisms/Treatment:	10
Loading:	1 organism/beaker
Feeding:	0.1 mL algal suspension and 0.24 mL digested Cerophyl®/test chamber daily
Lighting:	Fluorescent; 60-85 foot candles
Aeration:	Diluent water only prior to each renewal
Endpoints:	Mortality of adults; number of neonates produced in 3 broods
Water Quality:	Table A7-1

Results:

Survival

48-h Exposure:

DIMP caused a significant ($\alpha = 0.05$) decrease in Ceriodaphnia survival after 48-h of exposure to (Table A7-2). The 48-h LC50, as determined by Trimmed Spearman-Kärber method, is as follows:

48-h LC50 = 610 mg/L (95% confidence limits = 492.0 - 755.9)

7-d Exposure:

DIMP caused a significant ($\alpha = 0.05$) decrease in Ceriodaphnia survival after 7-d of exposure (Tables A7-3, A7-4, and A7-5). There was 0% survival in the 569 and 1138 mg/L concentrations after 7-d. A 7-d LC50, as determined by the Trimmed Spearman-Kärber method, is as follows:

7-d LC50 = 375 mg/L (95% confidence limits = 329.2 - 428.2)

NOAEL = 285 mg/L

LOAEL = 569 mg/L

Neonate Production

DIMP caused a significant ($\alpha = 0.05$) decrease in Ceriodaphnia neonate production after 7 d of exposure (Tables A7-3, A7-6, and A7-7). The 569 and 1138 mg/L concentrations were not included in the statistical analysis of neonate production because significant mortality occurred in these treatments.

NOAEL = 142 mg/L

LOAEL = 285 mg/L

TABLE A7-1. SUMMARY OF THE DIMP TOXICITY TEST WATER QUALITY DATA
FOR THE CLADOCERAN 7-DAY TEST - TEMPERATURE (°C)

	Test Concentrations (mg/L)					
	0	71	142	285	569	1138
<u>Day 0</u>						
0 H	25.0	25.0	25.0	25.0	25.0	25.0
<u>Day 1</u>						
0 H	25.0	25.0	25.0	25.0	25.0	25.0
24 H	24.9	24.9	24.8	24.9	24.9	24.9
<u>Day 2</u>						
0 H	25.0	25.0	25.0	25.0	25.0	25.0
24 H	24.8	24.9	24.9	25.0	24.8	24.8
<u>Day 3</u>						
0 H	25.0	25.0	25.0	25.0	25.0	25.0
24 H	24.9	24.9	25.0	24.8	24.9	24.9
<u>Day 4</u>						
0 H	25.0	25.0	25.0	25.0	25.0	
24 H	25.0	25.0	25.0	24.9	25.0	
<u>Day 5</u>						
0 H	25.0	25.0	25.0	25.0	25.0	
24 H	25.0	24.9	24.9	25.0	25.0	
<u>Day 6</u>						
0 H	25.0	25.0	25.0	25.0	25.0	
24 H	24.9	24.8	24.9	24.9		
<u>Day 7</u>						
24 H	24.9	24.9	25.0	25.0		
Mean	25.0	25.0	25.0	25.0	25.0	24.9
Min	24.8	24.8	24.8	24.8	24.8	24.8
Max	25.0	25.0	25.0	25.0	25.0	25.0

TABLE A7-1.(CONTINUED) - DISSOLVED OXYGEN (MG/L)

	Test Concentrations (mg/L)					
	0	71	142	285	569	1138
<u>Day 0</u>						
0 H	8.3	8.3	8.3	8.3	8.3	8.3
<u>Day 1</u>						
0 H	8.3	8.3	8.3	8.3	8.3	8.3
24 H	8.3	8.3	8.3	8.2	8.0	8.0
<u>Day 2</u>						
0 H	8.3	8.3	8.3	8.3	8.2	8.3
24 H	7.3	7.2	7.0	6.9	6.9	7.0
<u>Day 3</u>						
0 H	8.3	8.3	8.3	8.3	8.3	8.3
24 H	7.7	7.4	7.4	7.0	7.1	7.1
<u>Day 4</u>						
0 H	8.3	8.3	8.3	8.3	8.2	
24 H	7.8	7.6	7.6	7.3	7.2	
<u>Day 5</u>						
0 H	8.3	8.3	8.3	8.3	8.2	
24 H	7.9	7.7	7.6	7.4	7.0	
<u>Day 6</u>						
0 H	8.3	8.3	8.3	8.3		
24 H	7.8	7.8	7.6	7.3	6.9	
<u>Day 7</u>						
24 H	7.9	7.7	7.6	7.4		
Mean	8.1	8.0	7.9	7.8	7.7	7.9
Min	7.3	7.2	7.0	6.9	6.9	7.0
Max	8.3	8.3	8.3	8.3	8.3	8.3

TABLE A7-1. (CONTINUED) - pH (STANDARD UNITS)

	Test Concentrations (mg/L)					
	0	71	142	285	569	1138
<u>Day 0</u>						
0 H	7.16	7.20	7.20	7.19	7.22	7.29
<u>Day 1</u>						
0 H	7.18	7.26	7.21	7.25	7.22	7.24
24 H	7.09	7.19	7.20	7.15	7.01	7.17
<u>Day 2</u>						
0 H	7.10	7.13	7.17	7.21	7.18	7.24
24 H	7.05	6.98	6.94	6.89	6.91	6.97
<u>Day 3</u>						
0 H	7.14	7.19	7.20	7.24	7.19	7.23
24 H	7.10	7.02	6.99	6.91	6.94	7.00
<u>Day 4</u>						
0 H	7.12	7.15	7.17	7.20	7.14	
24 H	7.07	7.04	7.01	6.94	6.96	
<u>Day 5</u>						
0 H	7.09	7.13	7.17	7.23	7.17	
24 H	7.11	7.06	7.04	6.97	6.92	
<u>Day 6</u>						
0 H	7.14	7.11	7.18	7.20		
24 H	7.08	7.03	7.01	6.99	6.90	
<u>Day 7</u>						
24 H	7.10	7.07	7.03	7.01		
Min	7.05	6.98	6.94	6.89	6.90	6.97
Max	7.18	7.26	7.21	7.25	7.22	7.29

TABLE A7-1. (CONTINUED) - CONDUCTIVITY(μ MHOS/CM)

	Test Concentrations (mg/L)	
	0	1138
<u>Day 0</u>		
0 H	180	210
<u>Day 1</u>		
0 H	180	200
<u>Day 2</u>		
0 H	180	200
<u>Day 3</u>		
0 H	180	200
<u>Day 4</u>		
0 H	180	
<u>Day 5</u>		
0 H	190	
<u>Day 6</u>		
0 H	180	
<u>Day 7</u>		
24 H	180	
Mean	181	203
Min	180	200
Max	190	210

TABLE A7-1. (CONTINUED) - ALKALINITY (MG/L AS CaCO₃)

	Test Concentrations (mg/L)	
	0	1138
<u>Day 0</u>		
0 H	50	55
<u>Day 1</u>		
0 H	50	50
<u>Day 2</u>		
0 H	50	55
<u>Day 3</u>		
0 H	50	55
<u>Day 4</u>		
0 H	50	
<u>Day 5</u>		
0 H	50	
<u>Day 6</u>		
0 H	50	
<u>Day 7</u>		
24	55	
Mean	51	54
Min	50	50
Max	55	55

TABLE A7-1. (CONTINUED) - HARDNESS (MG/L AS CaCO₃)

	Test Concentrations (mg/L)	
	0	1138
<u>Day 0</u>		
0 H	60	68
<u>Day 1</u>		
0 H	55	64
<u>Day 2</u>		
0 H	60	68
<u>Day 3</u>		
0 H	60	64
<u>Day 4</u>		
0 H	55	
<u>Day 5</u>		
0 H	60	
<u>Day 6</u>		
0 H	55	
<u>Day 7</u>		
24 H	55	
Mean	58	66
Min	55	64
Max	60	68

TABLE A7-2. CLADOCERAN TOXICITY TEST DATA - SURVIVAL OF ADULTS AFTER
48 HOURS OF EXPOSURE TO DIMP

Concentration (mg/L)	Number Tested	Number Alive at 48 Hours	Percent Survival
Control	10	10	100
71	10	10	100
142	10	10	100
285	10	10	100
569	10	6	60
1138	10	0	0

TABLE A7-3. CLADOCERAN TOXICITY TEST DATA - SURVIVAL OF ADULTS,
TOTAL NUMBER OF YOUNG, AND NUMBER OF YOUNG PRODUCED
PER BROOD AFTER 7 DAYS OF EXPOSURE TO DIMP

Concentration (mg/L)	Rep	Brood No. 1	Brood No. 2	Brood No. 3	Total Young
Control	1	4	10	13	27
	2	4	9	12	25
	3	4	8	14	26
	4	3	8	12	23
	5	4	7	15	26
	6	4	9	14	27
	7	5	9	13	27
	8	4	9	14	27
	9	4	7	12	23
	10	3	10	16	29
71	1	5	7	14	26
	2	3	10	13	26
	3	3	9	11	23
	4	4	8	12	24
	5	4	9	15	28
	6	4	7	13	24
	7	5	9	11	25
	8	4	7	13	24
	9	5	8	14	27
	10	3	9	13	25

TABLE A7-3. (CONTINUED)

Concentration (mg/L)	Rep	Brood No. 1	Brood No. 2	Brood No. 3	Total Young
142	1	5	8	14	27
	2	4	7	12	23
	3	4	8	13	25
	4	3	9	12	24
	5	5	7	13	25
	6	5	9	14	28
	7	3	10	13	26
	8	4	8	12	24
	9	5	8	11	24
	10	3	9	12	24
285	1	3	6	11	20
	2	4	6	9	19
	3	4	7	9	20
	4	3	7	6	16
	5	3	5	DEAD	8
	6	5	7	12	24
	7	3	8	8	19
	8	4	6	6	16
	9	4	9	11	24
	10	5	8	10	23

TABLE A7-3. (CONTINUED)

Concentration (mg/L)	Rep	Brood No. 1	Brood No. 2	Brood No. 3	Total Young
569	1	DEAD			0
	2	DEAD			0
	3	DEAD			0
	4	DEAD			0
	5	DEAD			0
	6	0	0	DEAD	0
	7	DEAD			0
	8	DEAD			0
	9	DEAD			0
	10	DEAD			0
1138	1	DEAD			0
	2	DEAD			0
	3	DEAD			0
	4	DEAD			0
	5	DEAD			0
	6	DEAD			0
	7	DEAD			0
	8	DEAD			0
	9	DEAD			0
	10	DEAD			0

TABLE A7-4. CLADOCERAN DIMP TOXICITY TEST STATISTICAL ANALYSIS -
CLADOCERAN SURVIVAL AFTER 7 DAYS OF EXPOSURE

Data Transformation:

None

Shapiro-Wilk's Test for Normality:

This test could not be performed because total number of replicates was greater than 50.

Bartlett's Test for Homogeneity:

This test could not be performed because at least one group has zero variance.

Fisher's Exact Test:

Calculated test statistic:	See Table A7-5
Alpha value:	0.05
Critical value:	6
Conclusion:	Reject the null hypothesis that all groups are equal

TABLE A7-5. CLADOCERAN DIMP TOXICITY TEST STATISTICAL ANALYSIS -
RESULTS OF FISHER'S EXACT TEST ON SURVIVAL AFTER 7 DAYS
OF EXPOSURE

Concentration (mg/L)	Number Alive	Number Dead	b Value	Significance
Control	10	0		
71	10	0	10	
142	10	0	10	
285	9	1	9	
569	0	10	0	*
1138	0	10	0	*

* Significantly different at $\alpha = 0.05$ (Fisher's critical value = 6).

TABLE A7-6. CLADOCERAN DIMP TOXICITY TEST STATISTICAL ANALYSIS -
NEONATE PRODUCTION AFTER 7 DAYS OF EXPOSURE^a

Data Transformation:

None

Shapiro-Wilk's Test for Normality:

This test could not be performed because total number of replicates was greater than 50.

Steel's Many One Rank Test:

Calculated test statistic:

See Table A7-7

Alpha value:

0.05

Critical value:

77

Conclusion:

Reject the null hypothesis that all groups are equal

^a The 569 and 1138 mg/L treatments were not included in the statistical analysis of neonate production because significant mortality occurred in these treatments.

TABLE A7-7. CLADOCERAN DIMP TOXICITY TEST STATISTICAL ANALYSIS -
RESULTS OF STEEL'S MANY ONE RANK TEST ON NEONATE
PRODUCTION AFTER 7 DAYS OF EXPOSURE^a

Concentration (mg/L by Volume)	Mean Neonate Production	Rank Sum	Critical Value	df	Significance
Control	26.000				
71	25.200	91.00	77.00	10.00	
142	25.000	89.00	77.00	10.00	
285	18.900	60.00	77.00	10.00	*

^a The 569 and 1138 mg/L treatments were not included in the statistical analysis of neonate production because significant mortality occurred in these treatments.

* Significantly different at alpha = 0.05 (Critical values use k = 3).

APPENDIX 8

FATHEAD MINNOW ACUTE AND CHRONIC 7-DAY SURVIVAL AND GROWTH TEST CONDUCTED WITH DIISOPROPYL METHYLPHOSPHONATE (DIMP)

Test Method:	EPA/600/4-91/002 (Lewis et al., 1994)
Type of Test:	Static renewal (every 24 h)
Date:	July 8 -15, 1998
Investigator:	S. D. Turley
Laboratory:	UMD/WREC
Toxicant:	Diisopropyl methylphosphonate
Source:	Reagent grade (Purity > 99%)
Chemical Characteristics:	See Appendix 1
Dilution Water:	
Source:	20% Perrier:80% RO Water
Chemical Characteristics:	Table A8-1
Test Organism:	
Scientific Name:	<i>Pimephales promelas</i>
Age at Start of Test:	<24 h
Source:	UMD/WREC culture
Experimental Chambers:	
Material:	600 mL glass beaker
Test Solution Volume:	400 mL
No. Organisms/Replicate:	10
No. Organisms/Treatment:	40
Feeding:	Concentrated stock of 0.1 mL <i>Artemia</i> nauplii three times daily
Loading:	<0.5 g/L
Lighting:	Fluorescent; 60-85 foot candles
Aeration:	Prior to each renewal
Endpoints:	Mortality; growth
Water Quality:	Table A8-1

Results:

Mortality:

96-h Exposure:

Significant ($\alpha = 0.05$) mortality occurred in fathead minnow larvae exposed to 569 and 1138 mg/L DIMP for 96 h (Table A8-2). The 96-h LC50, as determined by the Trimmed Spearman-Kärber method, is as follows:

96-h LC50 = 604 (95% confidence limits = 542.1 - 672.8)

7-d Exposure:

Significant ($\alpha = 0.05$) mortality occurred to fathead minnow larvae exposed to 569 and 1,138 mg/L DIMP for 7 d (Tables A8-3, A8-4, and A8-5). The 7-d LC50, as determined by the Trimmed Spearman-Kärber method, is as follows:

7-d LC50 = 381 mg/L (95% confidence limits = 353.1 - 410.9)

NOAEL = 285 mg/L

LOAEL = 569 mg/L

Growth:

The growth of fathead minnow larvae was significantly ($\alpha = 0.05$) reduced by a 7-d exposure to 285, 569 and 1138 mg/L concentrations of DIMP for 7 d (Tables A8-3, A8-6, and A8-7). Fathead minnow larval growth was not affected by exposure to 142 or 71 mg/L concentrations of DIMP.

NOAEL = 142 mg/L

LOAEL = 285 mg/L

TABLE A8-1. SUMMARY OF THE DIMP TOXICITY TEST WATER QUALITY DATA
FOR THE FATHEAD MINNOW 7-DAY TEST - TEMPERATURE (°C)

	Test Concentrations (mg/L)	
	0	1138
<u>Day 0</u>		
0 H	25.0	25.0
<u>Day 1</u>		
0 H	25.0	25.0
24 H	24.9	24.9
<u>Day 2</u>		
0 H	25.0	25.0
24 H	24.8	24.8
<u>Day 3</u>		
0 H	25.0	25.0
24 H	24.9	24.9
<u>Day 4</u>		
0 H	25.0	
24 H	25.0	
<u>Day 5</u>		
0 H	25.0	
24 H	25.0	
<u>Day 6</u>		
0 H	25.0	
24 H	24.9	
<u>Day 7</u>		
24 H	24.9	
Mean	25.0	24.9
Min	24.8	24.8
Max	25.0	25.0

TABLE A8-1. (CONTINUED) - DISSOLVED OXYGEN (MG/L)

	Test Concentrations (mg/L)					
	0	71.1	142.3	284.5	569	1138
<u>Day 0</u>						
0 H	8.3	8.3	8.3	8.3	8.3	8.3
<u>Day 1</u>						
0 H	8.3	8.3	8.3	8.3	8.3	8.3
24 H	8.3	8.3	8.2	8.2	8.2	8.2
<u>Day 2</u>						
0 H	8.3	8.3	8.3	8.3	8.2	8.3
24 H	8.1	8.0	8.0	7.9	7.9	8.0
<u>Day 3</u>						
0 H	8.3	8.3	8.3	8.3	8.3	8.3
24 H	8.0	8.0	8.0	8.0	8.0	8.0
<u>Day 4</u>						
0 H	8.3	8.3	8.3	8.3	8.2	
24 H	8.0	8.1	8.1	8.0	8.0	
<u>Day 5</u>						
0 H	8.3	8.3	8.3	8.3	8.3	
24 H	8.0	8.0	8.0	8.0	8.0	
<u>Day 6</u>						
0 H	8.3	8.3	8.3	8.3	8.3	
24 H	8.0	8.1	8.0	8.0	7.9	
<u>Day 7</u>						
24 H	7.9	8.0	7.9	7.8	7.7	
Mean	8.2	8.2	8.2	8.1	8.1	8.2
Min	7.9	8.0	7.9	7.8	7.7	8.0
Max	8.3	8.3	8.3	8.3	8.3	8.3

TABLE A8-1. (CONTINUED) - pH (STANDARD UNITS)

	Test Concentrations (mg/L)					
	0	71.1	142.3	284.5	569	1138
<u>Day 0</u>						
0 H	7.16	7.20	7.20	7.19	7.22	7.29
<u>Day 1</u>						
0 H	7.18	7.26	7.21	7.25	7.22	7.13
24 H	7.20	7.13	7.13	7.12	7.13	7.18
<u>Day 2</u>						
0 H	7.10	7.13	7.17	7.21	7.18	7.24
24 H	7.00	6.97	6.96	6.99	6.99	7.04
<u>Day 3</u>						
0 H	7.14	7.19	7.20	7.24	7.19	7.23
24 H	7.11	7.03	6.98	7.01	7.04	7.09
<u>Day 4</u>						
0 H	7.12	7.15	7.17	7.20	7.14	
24 H	7.07	7.00	7.00	7.04	7.03	
<u>Day 5</u>						
0 H	7.09	7.13	7.17	7.23	7.17	
24 H	7.04	7.03	6.99	7.04	7.00	
<u>Day 6</u>						
0 H	7.08	7.11	7.18	7.20	7.16	
24 H	7.08	7.03	6.99	7.04	7.00	
<u>Day 7</u>						
24 H	7.15	7.09	7.00	7.09	7.06	
Min	7.00	6.97	6.96	6.99	6.99	7.04
Max	7.20	7.26	7.21	7.25	7.22	7.29

TABLE A8-1. (CONTINUED) - CONDUCTIVITY(μ MHOS/CM)

	Test Concentrations (mg/L)	
	0	1138
<u>Day 0</u>		
0 H	180	210
<u>Day 1</u>		
0 H	180	200
<u>Day 2</u>		
0 H	180	200
<u>Day 3</u>		
0 H	180	200
<u>Day 4</u>		
0 H	180	
<u>Day 5</u>		
0 H	190	
<u>Day 6</u>		
0 H	180	
<u>Day 7</u>		
24 H	180	
Mean	181	203
Min	180	200
Max	190	210

TABLE A8-1. (CONTINUED) - ALKALINITY (MG/L AS CaCO₃)

	Test Concentrations (mg/L)	
	0	1138
<u>Day 0</u>		
0 H	50	55
<u>Day 1</u>		
0 H	50	50
<u>Day 2</u>		
0 H	50	55
<u>Day 3</u>		
0 H	50	55
<u>Day 4</u>		
0 H	50	
<u>Day 5</u>		
0 H	50	
<u>Day 6</u>		
0 H	50	
<u>Day 7</u>		
24	50	
Mean	50	54
Min	50	50
Max	50	55

TABLE A8-1. (CONTINUED) - HARDNESS (MG/L AS CaCO₃)

	Test Concentrations (mg/L)	
	0	1138
<u>Day 0</u>		
0 H	60	68
<u>Day 1</u>		
0 H	55	64
<u>Day 2</u>		
0 H	60	68
<u>Day 3</u>		
0 H	60	64
<u>Day 4</u>		
0 H	55	
<u>Day 5</u>		
0 H	60	
<u>Day 6</u>		
0 H	55	
<u>Day 7</u>		
24	50	
Mean	57	66
Min	50	64
Max	60	68

TABLE A8-2. FATHEAD MINNOW DIMP TOXICITY TEST DATA - LARVAL SURVIVAL AFTER 96 HOURS OF EXPOSURE

Concentration (mg/L)	Rep	Number Tested	No. Alive at 96 Hours	Percent Survival
Control	1	10	10	100
	2	10	9	90
	3	10	10	100
	4	10	10	100
71	1	10	10	100
	2	10	10	100
	3	10	10	100
	4	10	9	90
142	1	10	10	100
	2	10	9	90
	3	10	10	100
	4	10	10	100
285	1	10	10	100
	2	10	10	100
	3	10	10	100
	4	10	10	100
569	1	10	1	10
	2	10	0	0
	3	10	0	0
	4	10	0	0
1138	1	10	0	0
	2	10	0	0
	3	10	0	0
	4	10	0	0

TABLE A8-3. FATHEAD MINNOW DIMP TOXICITY TEST DATA - LARVAL SURVIVAL AND GROWTH AFTER 7 DAYS OF EXPOSURE

Concentration (mg/L)	Rep	Number Larvae Alive	Percent Survival	Dry Weight ^a (mg)	Mean Dry Weight (mg)
Control	1	10	100	0.401	0.415
	2	8	80	0.395	
	3	10	100	0.454	
	4	10	100	0.408	
71	1	10	100	0.417	0.398
	2	10	100	0.434	
	3	9	90	0.380	
	4	9	90	0.362	
142	1	10	100	0.391	0.376
	2	8	80	0.328	
	3	10	100	0.415	
	4	10	100	0.370	
285	1	7	70	0.276	0.329
	2	8	80	0.321	
	3	9	90	0.346	
	4	10	100	0.374	
569	1	1	10	0.041	0.041
	2	0	0		
	3	0	0		
	4	0	0		
1138	1	0	0		0.000
	2	0	0		
	3	0	0		
	4	0	0		

^a Dry weight = Total dry weight of larvae/number of original larvae (10).

TABLE A8-4. FATHEAD MINNOW DIMP TOXICITY TEST STATISTICAL ANALYSIS
SURVIVAL OF LARVAE AFTER 7 DAYS OF EXPOSURE

Data Transformation:

Arc-sine square root

Shapiro-Wilk's Test for Normality:

Calculated test statistic:	0.92
Alpha value:	0.01
Critical value:	0.88
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity:

This test could not be performed because at least one group has zero variance.

Steels Many-One Rank Test

Calculated test statistic:	See Table A8-5
Alpha value:	0.05
Critical value:	10
Conclusion:	Reject the null hypothesis that all groups are equal

TABLE A8-5. FATHEAD MINNOW DIMP TOXICITY TEST STATISTICAL ANALYSIS -
RESULTS OF STEEL'S MANY-ONE RANK TEST ON LARVAL
SURVIVAL AFTER 7 DAYS OF EXPOSURE

Concentration (mg/L)	Mean Survival (%) ^a	Rank Sum	Critical Value	Significance
Control	95.0			
71	95.0	17.00	10.00	
142	95.0	18.00	10.00	
285	85.0	14.00	10.00	
569	42.5	10.00	10.00	*
1138	0.0	10.00	10.00	*

^a Values given are actual percent survival means rather than arc sine square root transformed means which were used in the statistical analysis.

* Significantly different at alpha = 0.05 (Steel's Critical value = 10.00).

TABLE A8-6. FATHEAD MINNOW DIMP TOXICITY TEST STATISTICAL ANALYSIS -
GROWTH OF LARVAE AFTER 7 DAYS OF EXPOSURE^a

Data Transformation:

No transformation

Shapiro-Wilk's Test for Normality:

Calculated test statistic:	0.94	
Alpha value:	0.01	
Critical value	0.84	
Conclusion:		Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	0.52	
Alpha value:	0.01	
Critical value:	11.34	
Conclusion:		Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	4.47	
Alpha value:	0.05	
Critical value:	3.49	
Conclusion:		Reject the null hypothesis that all groups are equal

Dunnett's Test:

Calculated test statistic:	See Table A8-7	
Alpha value:	0.05	
Critical value:	2.29	
Conclusion:		Reject the null hypothesis that the groups are equal

^a The 569 and 1138 mg/L DIMP concentrations were not included in the statistical analysis because significant mortality occurred after 7 days of exposure.

TABLE A8-7. FATHEAD MINNOW DIMP TOXICITY TEST STATISTICAL ANALYSIS
RESULTS OF DUNNETT'S TEST ON LARVAL GROWTH AFTER 7
DAYS OF EXPOSURE^a

Concentration (mg/L)	No. of Reps	Mean Dry Weight (mg) ^b	T Statistic	Significance
Control	4	0.415		
71	4	0.398	0.656	
142	4	0.376	1.555	
285	4	0.329	3.443	*

^a The 569 and 1138 mg/L treatments were not included in the statistical analysis of larval growth because significant mortality occurred in these treatments.

^b Values given are actual mean dry weights rather than arc sine square root transformed means which were used in the statistical analysis.

* Significantly different at alpha = 0.05 (Dunnett's critical value = 2.29).

APPENDIX 9

FROG EMBRYO TERATOGENESIS ASSAY (FETAX) - *XENOPUS* CONDUCTED WITH DIISOPROPYL METHYLPHOSPHONATE (DIMP)

Test Method:	ASTM Designation E 1439-91 ASTM (1998)
Type of Test:	Static renewal (every 24 h)
Date:	July 22 -26, 1998
Investigator:	S. D. Turley
Laboratory:	UMD/WREC
Toxicant:	Diisopropyl methylphosphonate
Source:	Reagent grade (Purity > 99%)
Chemical Characteristics:	See Appendix 1
Test Medium:	20% Perrier:80% RO Water
Test Organism:	
Scientific Name:	<i>Xenopus laevis</i>
Age at Start of Test:	Stage 8 blastula to stage 11 gastrula
Source:	UMD/WREC culture
Experimental Chambers:	
Material:	Glass petri dishes
Test Solution Volume:	10 mL
No. Organisms/Replicate:	25
No. Organisms/Treatment:	Control: 50 Groundwater: 50
Loading:	n/a
Lighting:	Fluorescent; 60-85 foot candles
Aeration:	Prior to renewals
Endpoints:	Mortality; malformation
Water Quality:	Table A9-1

Results:

Mortality:

The 96-h exposure to DIMP significantly ($\alpha = 0.05$) decreased frog embryo survival at concentrations of 569 mg/L and above (Table A9-2, A9-3, and A9-4). The 96-h LC50, as determined by the Trimmed Spearman-Kärber method, is as follows:

96-h LC50 = 1,543 mg/L (95% confidence limits = 1,357.3 - 1,753.2)

NOAEL = 398 mg/L

LOAEL = 569 mg/L

Malformations:

The 96-h exposure to DIMP did not cause a significant ($\alpha = 0.01$) decrease in the percentage of normally developing frog embryos at concentrations ranging from 71-398 mg/L (Tables A9-2 and A9-5). The concentrations above 398 mg/L were not used in the NOAEL/LOAEL malformation analysis because significant mortality occurred (Table A9-5). The 96-h EC50, as determined by the Trimmed Spearman-Kärber method, is as follows:

96-h EC50 = 1,225 mg/L (95% confidence limits = 1,028.1 and 1,459.5)

The types of malformed embryos are given in Table A9-6.

TABLE A9-1. SUMMARY OF THE DIMP TOXICITY TEST WATER QUALITY DATA
FOR FETAX - TEMPERATURE (°C)

	Test Concentrations (mg/L)									
	0	71	142	285	398	569	854	1138	2276	3414
<u>Day 0</u>										
0 H	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0
<u>Day 1</u>										
0 H	22.9	22.9	22.9	23.0	22.9	23.0	22.9	23.0	22.9	22.9
<u>Day 2</u>										
0 H	23.0	23.0	23.0	23.0	23.0	22.9	23.0	22.9	23.0	23.0
<u>Day 3</u>										
0 H	22.9	22.9	22.9	22.9	22.9	22.9	23.0	23.0	23.0	23.0
<u>Day 4</u>										
24 H	23.0	23.0	22.9	22.9	23.0	22.9	23.0	23.0	23.0	22.9
Mean	23.0	23.0	22.9	23.0	23.0	22.9	23.0	23.0	23.0	23.0
Min	22.9	22.9	22.9	22.9	22.9	22.9	22.9	22.9	22.9	22.9
Max	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0

TABLE A9-1. (CONTINUED) - pH (STANDARD UNITS)

	Test Concentrations (mg/L)									
	0	71	142	285	398	569	854	1138	2276	3414
<u>Day 0</u>										
0 H	7.60	7.57	7.57	7.55	7.56	7.54	7.52	7.50	7.49	7.45
<u>Day 1</u>										
0 H	7.66	7.65	7.66	7.64	7.62	7.59	7.57	7.55	7.56	7.51
<u>Day 2</u>										
0 H	7.67	7.66	7.63	7.62	7.60	7.57	7.55	7.52	7.50	7.47
<u>Day 3</u>										
0 H	7.64	7.63	7.62	7.60	7.59	7.57	7.55	7.52	7.52	7.48
<u>Day 4</u>										
24 H	7.70	7.69	7.67	7.66	7.63	7.60	7.57	7.55	7.55	7.51
Min	7.60	7.57	7.57	7.55	7.56	7.54	7.52	7.50	7.49	7.45
Max	7.67	7.66	7.66	7.64	7.62	7.59	7.57	7.55	7.56	7.51

TABLE A9-2. FETAX TOXICITY TEST DATA - PERCENT EMBRYO SURVIVAL AND MALFORMATIONS AFTER 96 HOURS OF EXPOSURE TO DIMP

Concentration (mg/L)	Rep	Number Embryos Alive	Percent Survival	Number Embryos Malformed	Percent Malformed
Control	1	24	96	2	8.3
	2	25	100	2	8.0
71	1	24	96	2	8.3
	2	24	96	3	12.5
142	1	23	92	2	8.7
	2	24	96	3	12.5
285	1	23	92	2	8.7
	2	24	96	4	16.7
398	1	23	92	3	13.0
	2	22	88	7	31.8
569	1	23	92	9	39.1
	2	21	84	6	28.6
854	1	22	88	7	31.8
	2	21	84	7	33.3
1138	1	17	68	7	41.2
	2	18	72	7	38.9
2276	1	10	40	7	70.0
	2	9	36	7	77.8
3414	1	0	0	0	DEAD
	2	1	4	1	100.0

TABLE A9-3. FETAX DIMP TOXICITY TEST STATISTICAL ANALYSIS - PERCENT EMBRYO SURVIVAL AFTER 96 HOURS OF EXPOSURE

Data Transformation:

Arc-sine square root

Shapiro-Wilk's Test for Normality:

Statistic not run because only two replicates were used in the test as recommended in the test protocol. The data were assumed to be normally distributed. See Section 2.5 in the body of the report for further details.

Bartlett's Test for Homogeneity of Variances:

Statistic not run because only two replicates were used in the test as recommended in the test protocol. Homogeneity of variance was assumed to occur. See Section 2.5 in the body of the report for further details.

ANOVA

Calculated test statistic:	65.58
Alpha value:	0.05
Critical value:	3.02
Conclusion:	Reject the null hypothesis that all groups are equal

Dunnett's Test

Calculated test statistic:	See Table A9-4
Alpha value:	0.05
Critical value:	2.81
Conclusion:	Reject the null hypothesis that all groups are equal

TABLE A9-4. FETAX DIMP TOXICITY TEST STATISTICAL ANALYSIS - RESULTS OF DUNNETT'S TEST ON PERCENT EMBRYO SURVIVAL AFTER 96 HOURS OF EXPOSURE

Concentration (mg/L)	Number of Reps	Mean Survival (%) ^a	T Statistic	Significance
Control	2	98		
71	2	96	0.614	
142	2	94	1.341	
285	2	94	1.341	
398	2	90	2.435	
569	2	88	2.850	*
854	2	86	3.332	*
1138	2	70	6.159	*
2276	2	38	10.861	*
3414	2	2	18.237	*

^a Values given are actual percent survival means rather than arc sine square root transformed means which were used in the statistical analysis.

* Significantly different at alpha = 0.05 (Dunnett's critical value = 2.81).

Table A9-5. FETAX DIMP TOXICITY TEST STATISTICAL ANALYSIS - PERCENT MALFORMATIONS AFTER 96 HOURS OF EXPOSURE^a

Data Transformation:

Arc-sine square root

Shapiro-Wilk's Test for Normality:

Statistic not run because only two replicates were used in the test as recommended in the test protocol. The data were assumed to be normally distributed. See Section 2.5 in the body of the report for further details.

Bartlett's Test for Homogeneity of Variances

Statistic not run because only two replicates were used in the test as recommended in the test protocol. Homogeneity of variance was assumed to occur. See Section 2.5 in the body of the report for further details.

ANOVA

Calculated test statistic:	5.19
Alpha value:	0.05
Critical value:	1.46
Conclusion:	Fail to reject the null hypothesis that all groups are equal

^a The concentrations of 569, 854, 1138, 2276, and 3414 mg/L were not included in the statistical analysis of malformations because significant mortality occurred by 96 h.

TABLE A9-6. FETAX DIMP TOXICITY TEST DATA - TYPE AND NUMBER OF MALFORMED EMBRYOS AFTER 96 HOURS EXPOSURE

Malformation	Test Concentration (mg/L)																				Tot No.	
	0		71		142		285		398		569		854		1138		2276		3414			
	Rep		Rep		Rep		Rep		Rep		Rep		Rep		Rep		Rep		Rep			
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2		
Severe							1						1	1			1	1	1	1	1	8
Gut coiling		1				1		1	1	1	2	4	2	3	1	3	2	2	1			25
Edema:																						0
Multiple	2	1	2	3	1	3		3	2	3		3	2	2	4	3	2	4	4			44
Cardiac												1										1
																						0
Abdominal																						
Facial																						0
Cephalic																						0
Blisters																						0
Tail																						0
Notochord											1	1	1	1	2		1		1			8
Fin																						0
Face																	1					1
Eye											1*											1
Brain																						0
Hemorrhage																						0
Cardiac																						0
Other																						0
Total No.	2	2	2	3	2	3	2	4	3	7	9	6	7	7	7	7	7	7	0	1		88

* Embryo missing eye.